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# THE HISTOLOGICAL LESIONS OF ACUTE GLANDERS IN MAN AND OF EXPERIMENTAL GLANDERS IN THE GUINEA-PIG.

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PLATES XIX AND XX.

## I. A CASE OF HUMAN GLANDERS.

ON account of the rarity of glanders infection in man in this country, and in view of the apparent uncertainty which exists regarding the histology of the lesions of the disease, the following case is of interest:

*Clinical History.*—The patient, an adult male, entered the Malden (Massachusetts) Hospital October 24, 1895, in the service of Dr. J. Richmond Barss, with a gangrenous condition of the middle finger and extensive suppuration in the hand. His history, which was obtained later, was as follows: On September 30th, while skinning a farcy horse, he cut his finger. The next day his finger pained him, but did not appear to be injured. The pain continued, and he took to his bed, where he was seen by a physician eight days later, who diagnosed a felon and incised the finger. He remained in bed with fever and great thirst until October 24th, when he was admitted to the hospital. His temperature some hours after entrance was 105° F. The next day amputation of the finger at the metacarpo-phalangeal articulation was performed.

About the fourth day after admission pustules appeared on the forehead and upon other parts of the body. Later those on the forehead became confluent and underwent ulceration, while symptoms of marked septic poisoning developed, the temperature reaching 105° F. There were no nasal symptoms. Death occurred November 1st, one week after entrance into the hospital and thirty-one days after receiving the wound in the finger.

*Autopsy.*—Five hours after death. The body is that of a well-built, muscular man. Subcutaneous fat moderate in amount. Rigor mortis very marked. Slight lividity of dependent portions. Amputation of finger.

The skin of the forehead as low as the eyebrows and of the scalp as far back as the vertex is thickened, discoloured, eroded, and feels dense to the touch. The margin of this area is escalloped, and is rather sharply elevated above the adjoining normal skin. In colour it is purple, mottled with small yellow areas. Over the upper portion of the forehead and at the beginning of the scalp the epidermis is purple to black in colour, and more or less detached from the underlying tissue, from which it can be easily removed. In places it is lacking, leaving irregular and rather superficial ulcerations, in which there can be made out many yellow specks, not soft enough to be called pus. On section the tissue is deeply infiltrated with an opaque, yellowish-white material, which is rather firm, and can not be squeezed out like pus. In the left upper eyelid are several pustules, single and grouped. Over the zygomatic arch and on the right side there are also two small groups of pustules. In the skin of the left arm, on its outer and posterior surfaces, are scattered several pustules from one to three millimetres in diameter and about one millimetre in elevation. Three or four similar pustules are seen on the skin of the right arm, and one on the skin over the tibia. On the front of the chest are two pustules, besides a small group below the outer end of the left clavicle. The pustules found in these situations generally present a purplish tint about their bases. In the pectoral muscle on the right side, near the sterno-clavicular articulation, is a small, oval, yellow, semi-solid nodule about the size of a split pea. The left axilla contains an area of suppuration in the axillary fatty tissue about 5 centimetres long and 3 centimetres in diameter. On section, this area resembles a bit of very coarse sponge, the meshes of which are filled with a thin, odourless, yellowish fluid. Some of the lymphatic glands in the neighbourhood are still well preserved, although considerably enlarged, and on section they show a yellow infiltration on the side toward the pus.

*Lungs.*—The left lung over the upper lobe is bound down by fibrous

adhesions. The right lung is free. Both are moderately congested, and slightly œdematous posteriorly. On the lower surface of the right upper lobe are two yellow, elevated areas, 2 millimetres in diameter, surrounded by a dark-red zone. The pleura over both areas is covered with a thin coat of fibrin. Scattered through the remainder of the right lung, usually just beneath the pleura, are in all about a dozen small resistant areas, the largest not more than 1 centimetre in diameter. On section, these areas are sometimes found to be solid, of a dark-red colour, with yellow points; sometimes softened or broken down, forming abscesses or cavities filled with fluid of a purulent character. On the pleura over these areas there is often a thin fibrinous exudate.

*Heart.*—Not remarkable.

*Spleen.*—Enlarged to half again its normal size; soft. Follicles not visible on section.

*Kidneys.*—Capsule very slightly adherent. On section, cortex more opaque than normal. Pyramids injected.

*Liver.*—Slight cloudy swelling.

The deep cervical and mesenteric lymphatic glands slightly enlarged and congested.

The nasal cavities and pharynx were not examined.

Bacteriological Examination.—Cover-glass preparations from the lesions on the scalp and from the pus of the axillary abscess showed the presence of a few bacilli of medium size, with rounded ends, varying in length, and having faintly staining spaces in the protoplasm. The purulent-looking fluid from a small abscess cavity in the lung was negative on cover-glass examination. Cultures were made on coagulated blood serum (Löffler's mixture) in test tubes from the various lesions and from the organs, with the following results, after about thirty-six hours in the incubator:

*Pus from the Axillary Abscess.*—The surface of the culture medium was thickly sown with discrete and confluent colourless colonies, resembling small drops of dew, and varying in diameter from a pin point to about 1.5 millimetre. The growth was slow, the colonies being barely visible after twenty-four hours.

*Cutaneous Lesions.*—Cultures were made from four different places, including the ulcerated tissue of the scalp and the pustules, with the following results:

Culture No. 1: Very numerous yellow colonies and a few minute

colourless colonies. The former were colonies of the staphylococcus pyogenes aureus.

Culture No. 2: A few small colourless colonies and numerous colonies of the staphylococcus pyogenes aureus.

Culture No. 3: Very numerous discrete and confluent colourless, wet-looking colonies, of the same appearance as those in the culture from the axillary abscess.

Culture No. 4: Rather numerous colourless, moist-looking colonies, the largest about 1 millimetre in diameter. A very few contaminating colonies present.

*Abscess of Lung.*—Only three or four colonies developed. These were small, colourless and moist-looking, the largest about 1 millimetre in diameter.

*Spleen.*—Half a dozen of the same colourless, moist-looking colonies.

*Kidney.*—One colony of an unknown organism.

*Liver.*—Some unknown bacteria.

*Blood of the Heart.*—Sterile.

The colourless colonies which were present in the cultures from the pus of the axillary abscess, cutaneous lesions, abscess of the lung, and the spleen, were found to be composed of one and the same organism—namely, a bacillus of medium size, with rounded ends and of variable length. It was usually short, but sometimes appeared in long forms, which were slightly wavy or curved. Its protoplasm frequently showed irregularly distributed, faintly staining areas. The shorter forms occurred frequently in pairs, and showed an ill-defined faintly stained space in the centre of the rod. The colonies on blood serum, if well separated, attained after a time a diameter of several millimetres. At first they were colourless, translucent, and viscid-looking, but later they assumed a brown tint. Pure cultures were obtained on potatoes of the bacilli from all of the original cultures. The appearances of all these potato cultures were the same. After thirty-six hours in the incubator the growth appeared in the form of a moist, translucent, viscid-looking material. Later this acquired a brown colour, and resembled honey in appearance. After a few days it became a darker brown, lost its translucency, and the potato around took on a dark-gray colour. The growth of the bacilli in cultures was slow, thirty-six hours being required for a fair development.

The morphological and cultural peculiarities above described are identical with those of the bacillus of glanders.

Results of the Inoculation of Guinea-pigs.—Guinea-pigs were inoculated with young blood-serum cultures of the bacilli derived from each of the original cultures. In inoculating, either a suspension in sterilized salt solution was injected with a hypodermic syringe or the material from the cultures was introduced beneath the skin through a wound by means of a platinum wire. The quantity of the suspension injected was not more than about 0.5 cubic centimetre for each animal, except in the case of one which received 1 cubic centimetre. The chief results of these inoculations were as follows:

BACILLI FROM THE AXILLARY ABSCESS.

Guinea-pig No. 1: Subcutaneous inoculation in abdominal wall. Death after twenty-one days. Ulcer at point of inoculation; abscess of scrotum; abscess of inguinal lymphatic gland; abscess of anterior nares; small nodules in spleen and liver.

Guinea-pig No. 2: Inoculation into peritoneal cavity. Death in forty-eight hours. Nodules in testicular membrane; fibrinous peritonitis, with minute grayish granulations; adrenal glands and lymphatic glands hæmorrhagic.

BACILLI FROM CUTANEOUS LESIONS.

Culture No. 1: Subcutaneous inoculation in abdominal wall. Death after twenty-four days. Ulcer at point of inoculation; ulcer of scrotum; abscess of testicle; abscess of inguinal lymphatic gland; nodules in spleen and liver.

Culture No. 2: Subcutaneous inoculation in left thigh. Ulcer at point of inoculation. Killed after eight days. No characteristic lesions.

Culture No. 3; Guinea-pig No. 1: Subcutaneous inoculation in abdominal wall. Killed after sixteen days. Ulcer at point of inoculation; yellow nodules beneath membranes of testicles; abscess of dorsum involving vertebræ.

Guinea-pig No. 2: Subcutaneous inoculation both in abdominal wall and in thorax. Death after fourteen days. Ulcer at point of inoculation; nodules in testicle and in spleen.

Culture No. 4: Inoculation in peritoneal cavity. Death in four days and a half. Œdema and congestion of scrotum; purulent infiltration of membranes of testicle; localized fibrinous peritonitis; nodules in spleen.

BACILLI FROM THE ABSCESS OF LUNG.

Guinea-pig No. 1: Inoculation into peritoneal cavity. Death in thirty-six hours. Small nodules in omentum, liver, and spleen; lymphatic glands hæmorrhagic.

Guinea-pig No. 2: Death after twenty-two days. Abscess of testicle; abscess of anterior nares; suppuration about joints involving the bone; abscess of inguinal lymphatic glands; nodules in liver; large nodule in spleen.

BACILLI FROM THE SPLEEN.

Guinea-pig No. 1: Inoculation in the peritoneal cavity. Death in thirty-six hours. Small nodules in peritonæum, spleen and liver; omentum infiltrated with fibrino-purulent exudation; lymphatic glands hæmorrhagic.

Guinea-pig No. 2: Subcutaneous inoculation in abdominal wall. Culture derived from the spleen of guinea-pig No. 1. Death after twelve days. Ulcer at point of inoculation; ulcer of scrotum; adhesive inflammation of membranes of testicles, with formation of yellow foci.

Summing up the results of the animal inoculations, it may be said that the bacilli found in at least six of the seven original cultures produced the characteristic lesions of experimental glanders in the guinea-pig—namely, the acute inflammatory lesions of the scrotum or testicle and the grayish, tuberclelike nodules in various situations. The negative result in the case of the bacilli from one of the original cultures may be explained by the fact that the animal was killed before sufficient time had elapsed for metastases to take place, the bacilli at the time of the killing of the animal being still localized at the point of inoculation. The organism inoculated into the animal was identical in morphology and cultural peculiarities with those of the other cultures. The bacteriological examination of the case, therefore, showed the presence of a bacillus in the axillary abscess, in the lesions of the skin and lungs, and in the spleen, which, on account of its morphology, mode of growth upon potatoes and pathogenic effects on guinea-pigs, must be considered to be the bacillus of glanders.

Two guinea-pigs were also inoculated respectively with the pus of the axillary abscess and with the purulent fluid from a small ab-

scess or broken-down nodule in the lung, from which a few colonies of glanders bacilli were cultivated. The inoculations were made into the peritoneal cavity by incision. The animal inoculated with the pus of the axillary abscess showed swelling of the scrotum, and was killed after seventy-two hours. At autopsy, yellowish nodules were found in the testicles and a few similar nodules in the peritonæum. The animal inoculated with the purulent fluid from the lung nodule was killed after fifteen days. The scrotum was markedly swollen and the animal emaciated. The testicles contained caseous areas, and the membranes were infiltrated with inflammatory exudation, which also involved the scrotal tissues.

A number of cultures made from the inoculated animals resulted as follows: The bacillus was found in the liver in nine out of thirteen animals; in the spleen in eleven out of thirteen; in the kidney in two out of eleven; and in the blood of the heart in three out of eleven. The bacilli in the blood of the heart were few, and the cases in which they were found in that situation were rapidly fatal. In the kidney the bacilli were also present in but small numbers, whereas they were sometimes numerous in the spleen and liver.

A number of the lesions in the testicle and many of the areas of abscess formation in the lymphatic glands and elsewhere contained the bacilli in large numbers in apparently pure culture except in one instance.

Histological Examination.—Sections were prepared from the lesions of the skin, the axillary abscess, lymphatic glands, lesions of the lungs, nodule in pectoral muscle, and from the kidney, spleen, and liver. For hardening, Zenker's fluid or alcohol, or both, were employed.

The stains used were hæmatoxylin with eosin, and fuchsin with aurantia. No satisfactory method of staining the bacilli in sections was found. The results of the study of these sections were as follows:

*Lesions of the Scalp.*—Under the low power the subcutaneous tissue seems to be more or less densely infiltrated with a purulent-looking material, containing very numerous small, deeply staining granules. In places some of the structure of the tissue is preserved;

in other areas no vestige of the original tissue remains, but foci are present which are made up of the above-mentioned material. Large lymph vessels near the muscular layer are seen filled with the same substances. The tissue immediately beneath the epidermis contains a large amount of blood. The epidermis in places is more or less disintegrated, the upper layer being separated from the lower layers by loose cells and detritus. The tissue beneath the muscle contains a large amount of fibrin and rather numerous nuclei.

Under the high power the infiltrating material resolves itself into polynuclear leucocytes, deeply stained chromatin fragments of varying size and shape, faintly stained nuclei, red blood-corpuscles, the empty membranes of dead cells or the skeletons of nuclei, together with necrotic detritus. The leucocytes and chromatin fragments in varying proportions constitute the greater part of the mass. The chromatin fragments vary in form and size from small granules to oval and irregular masses of the size of large cells. In the tissue beneath the muscular layer the nuclei observed with the low power are seen to belong to polynuclear leucocytes and to cells which belong to apparently proliferated lymphatic endothelium. The latter cells have oval vesicular nuclei and considerable cytoplasm. Polynuclear leucocytes are also seen in places between the fibres of the muscular layer.

In the disintegrated portions of the epidermis are seen many epithelial cells in all stages of disintegration, some polynuclear leucocytes and a few red blood-corpuscles, besides rather numerous delicate unstained ringlike bodies. These last vary in size from something less than that of a red blood-corpuscle to that of a polynuclear leucocyte, or are even larger. Some of them have a contracted and irregular shape, suggesting the membranes of dead cells. The origin of these bodies is not clear. Some of them apparently represent degenerated red blood-corpuscles.

A large pustule on the face presented essentially the same conditions as were observed in the more diffuse lesions of the scalp.

In a section through a small pustule 3 or 4 millimetres in diameter the corium and the epidermis, except the horny layer, have disappeared, and in the place of these are seen polynuclear leucocytes and

cellular detritus, besides a few red blood-corpuscles and chromatin bodies. The neighbouring tissue for some distance is infiltrated with the same material. The pea-sized nodule in the pectoral muscle on section appeared under the low power as a necrotic-looking material. Under the high power this was seen to contain many leucocytes, frequently showing degenerative appearances and much cellular detritus. Masses of a reticulated substance resembling fibrin were also present, as well as many deeply staining granules and globules of chromatin.

*Pulmonary Lesions.*—A number of these were studied in sections. The sections of nodules or consolidated areas showed the alveoli in the peripheral portions to be filled with a material consisting of leucocytes, red blood-corpuscles, and fibrin, together with proliferated alveolar epithelium. These elements were present in varying proportions in different places and in different sections. In some parts of the consolidated areas—particularly in the central portions—the walls of the alveoli have more or less disappeared, and have been replaced by a necrotic-looking material, containing numerous polynuclear leucocytes and some chromatin fragments. Small bronchi, devoid of epithelium, may be seen filled with this material. Sections of consolidated areas which have undergone extensive softening, and in some places are broken down, and of cavities containing fluid and detritus, were also studied. The softened material and the remains of the contents of the cavities were found to consist essentially of necrotic and degenerated cells, together with leucocytes in larger or smaller numbers. In one instance there was granulation tissue in the wall of the cavity. The histogenesis of the lesions in the lung seems to be the filling up of the alveoli with inflammatory exudation and proliferated epithelial cells followed by degeneration and necrosis in the area of consolidation so formed, resulting eventually in cavity formation. The process is essentially a focal pneumonia, with suppuration and necrosis.

*Axillary Abscess.*—The tissue involved showed an infiltration with large oval cells with vesicular nuclei, which were apparently derived from the lymphatic endothelium. These cells were often more or less degenerated, and in places formed nothing more than ne-

crotic detritus containing leucocytes. The necrotic material seems to represent the pus of the abscess.

*Lymphatic Glands.*—In the lymphatic glands in the neighbourhood of the axillary abscess it was possible to demonstrate proliferation of the endothelium of the lymph sinuses and coarse strands of fibrin in the blood vessels. Sections of other lymphatic glands presented no remarkable lesion.

*The Kidneys.*—Sections hardened in Zenker's fluid, and stained with fuchsin and aurantia, gave the following results: The epithelial cells of the cortical tubules are very granular, often separated from one another and projecting far into the lumen of the tubule. Many nuclei are stained more deeply and homogeneously than normal; others stain only faintly, and are more or less shrunken. In the lumina of most of the tubules a peculiar material is seen. This usually appears in the form of delicate rings or circles closely packed together, along with which are red-stained granules resembling chromatin. These rings vary in diameter from about that of a red blood-corpuscle to that of a large nucleus of the renal epithelium. They are often distorted or irregular in outline. In some tubules the material has the appearance of an indefinite granular detritus staining more or less red. Material similar to that in the tubules is also to be seen in small amounts in the capsular spaces of the glomeruli. Its origin is obscure. Some of it may represent degenerated red blood-globules, but the study of the sections gave little evidence that such was the case. That it is the remains of degenerated and extruded nuclei is also suggested, but there does not seem to be a sufficient diminution in the number of nuclei in the renal epithelium to account for the great amount of it which is demonstrable. It is apparently the same as that seen in the renal tubules of the guinea-pig, as shown in Plate XX, Fig. 4.

*The spleen* showed the presence of a large amount of blood in the sections. *The liver* was not remarkable.

## II. The Histological Lesions of Experimental Glanders in the Guinea-pig.

At the present time the generally accepted ideas of the histology of the glanders tubercle and of the histogenesis of its formation are those which were advanced by Baumgarten. This investigator states in his well-known "Lehrbuch der pathologischen Mykologie" that the histological composition of the experimental glanders tubercle and that of the miliary tubercle are essentially the same. He holds that the glanders tubercle, like the miliary tubercle, at first consists of epithelioid cells, among which leucocytes later appear, but that the two lesions differ mainly in the fact that the glanders tubercle undergoes necrotic and suppurative changes more quickly than the miliary tubercle. The epithelioid cells are, according to his observations, derived from the fixed tissue cells. According to Baumgarten, therefore, the primary effect of the bacillus of glanders upon a tissue is a new growth of cells with secondary degenerative changes rather than necrosis and suppuration from the beginning.

The evidence which Baumgarten offers in support of his views is not convincing. He states that he has seen epithelioid cells in glanders tubercles in mice and guinea-pigs; but his drawing of a section of a small glanders focus in the liver of a mouse would seem to show that what he considered to be epithelioid cells were none other than degenerated liver cells.

Since Baumgarten, Tedeschi* is apparently the only writer who has investigated the histology of the lesions of experimental glanders. He says that his observations have not enabled him to confirm the opinions of Baumgarten, but rather support the view that the primary effect of the bacillus of glanders upon a tissue is necrosis and the invasion of leucocytes. The difference between his results and those of Baumgarten, he suggests, may be due to the greater virulence of his cultures. Tedeschi's descriptions, however, of the histology of the lesions encountered in various situations are not very definite or altogether satisfactory.

* Ziegler's *Beiträge z. path. Anat.*, Bd. xiii, 1893.

From what has been said it is evident that the histology and histogenesis of the experimental glanders tubercle are still not clearly understood, and it is as a contribution to this subject that the following observations are offered:

*Methods.*—For the study of the histology of the lesions of experimental glanders paraffin sections of the tissues of nine of the inoculated guinea-pigs were prepared. Most of these tissues were hardened in both Zenker's fluid and in alcohol. The duration of the infection in the nine animals was thirty-six hours in the case of two, and forty-eight hours, four days and a half, fourteen, sixteen, twenty-one, twenty-two, and twenty-four days respectively in the remaining seven animals. The material for the study comprised sections of the livers, spleens, lungs, adrenals, and lymphatic glands of the nine guinea-pigs, of the kidneys of eight, of the testicles of seven, and of the peritoneal exudates from three.

The most satisfactory method of staining sections of tissue hardened in Zenker's fluid was found to be double staining with fuchsin and aurantia. The fuchsin was used in a solution made up of about 10 cubic centimetres of the saturated alcoholic solution with 100 cubic centimetres of water. Of aurantia an aqueous solution something below one per cent was employed. The demonstration of the bacilli in sections was found to be very difficult. Unless they were numerous, they could rarely be found. No satisfactory method for the staining of the bacilli in sections was arrived at, although many experiments with various methods were made with this end in view.

*Liver.*—This organ frequently presented minute grayish points and sometimes yellow areas. In the sections examined these yellow areas seemed to correspond to areas of necrosis of liver tissue, and the smaller grayish points to small glanders tubercles or foci. Under the low power the glanders tubercles appeared as foci or aggregations of small bodies of varying size and shape, staining deeply with fuchsin, and resembling at first sight collections of leucocytes. These foci were observed in the sections of eight out of the nine livers, usually in small numbers. Their size varied from about that of the area occupied by half a dozen liver cells upward. Under a high power they

were seen to consist of bodies staining deeply with fuchsin, polynuclear leucocytes, structures looking like the skeletons of dead nuclei of various kinds, and an indefinite material, apparently the remains of dead cells. A small glanders tubercle under a high power is shown in Plate XIX, Fig. 1. The deeply staining bodies were the most striking objects in the areas, and from their appearance they were evidently of the nature of chromatin. In shape and size they were very variable. Some were round, and ranged in size from that of a minute globule to that of a red blood-corpuscle. Some were large, elongated, club-shaped, or constricted at the middle. Others were irregular or lobulated, as if from the fusion of several of the round forms. All were homogeneous and stained deeply. The smallest foci consisted essentially of broken-down liver cells, polynuclear leucocytes, tissue detritus, and a few of the chromatin fragments.

From a study of these areas in the liver it seems that the process of their formation consists in the degeneration and destruction of liver tissue and the invasion of polynuclear leucocytes, together with changes in the nuclei of the tissue cells and of the leucocytes, whereby the chromatin bodies are produced. There was no evidence of the production of the epithelioid cells of Baumgarten's description.

In addition to these lesions, other changes in the liver were met with. Among the more prominent was the occurrence of liver cells, isolated or in groups, whose nuclei were stained much more deeply and homogenously than usual (Plate XIX, Fig. 1), while the cytoplasm in some cases was also denser and more deeply stained. This deep staining of the nuclei was also seen in the areas of necrosis mentioned above, and in some instances about the glanders foci. In the bile ducts in some cases a granular material could be seen which, on close examination under a high power, was seen to consist mainly of delicate rings of about the diameter of the nuclei of the epithelial cells of the duct. Adherent to these rings or among them were minute fuchsin-stained granules. Similar indefinite, irregular rings, with red-stained granules, were also seen in some instances in the capillaries and lymph spaces. These, as well as the material in the bile ducts, suggested degenerated nuclei.

*Spleen.*—The presence of minute grayish nodules in the spleen in experimental glanders is very characteristic of the disease. In the sections from eight spleens small glanders foci were present in varying numbers. They did not occupy any special situation in the organ. Under the low power these foci appeared as aggregations of small deeply stained granules. Under the high power essentially the same elements were seen as in the foci in the liver. Chromatin bodies, of varying size and shapes, polynuclear leucocytes, faded-out nuclei, and cellular detritus were recognised in the material of which the area was composed. In some cases fibrin was also present. Sometimes the nuclei of the tissue immediately surrounding a focus stain faintly, as if undergoing degeneration. The smallest areas show the deeply staining bodies and a few leucocytes, together with a fading out or disappearance of the nuclei and a breaking up of the cytoplasm of the cells. As in the liver, the process seems to be one of necrosis and invasion by leucocytes, accompanied by certain degenerative changes in the nuclei, in consequence of which the chromatin bodies are formed.

In some cases masses of fibrin could be demonstrated in the sinuses by means of Weigert's fibrin stain.

*Lungs.*—In the sections from seven animals more or less well-developed glanders foci were found. These consisted essentially of the same material as was found in the foci in the livers and spleens—namely, numerous deeply red-staining bodies of variable size and shape, leucocytes, and cellular detritus. Unstained or faintly stained bodies, looking like faded and distorted nuclei of tissue cells and leucocytes, together with irregular ringlike structures resembling the empty membranes of dead cells, were also seen. In some instances the walls of the air cells were preserved to some extent; in others, they were not to be made out. From a study of the smaller, or what were considered as beginning foci, it would seem that these focal lesions are due to a proliferation of the epithelium of the air cells and the invasion of leucocytes, together with degenerative processes in these elements by which the chromatin bodies are formed.

In the bronchi a peculiar material was frequently present in large

amounts. This consisted mainly of delicate rings or circles, sometimes irregular, and varying in diameter, the largest being about the size of the nucleus of a bronchial epithelial cell. Minute fuchsin-staining granules were adherent to and also scattered among these rings. This material was similar to that observed in the bile ducts of the liver. The appearances of these rings suggested that they represented dead and extruded nuclei from the epithelium of the bronchus, but the absence of any marked diminution in the numbers of these nuclei seemed to negative this idea. That some of them at least may be degenerated red blood-corpuscles seems possible.

*Lymphatic Glands.*—The lymphatic glands, especially in the rapidly fatal cases, were often enlarged and red. In the more chronic cases they were usually enlarged, and sometimes showed caseous areas or abscess formation. Sections of lymphatic glands from nine animals were studied. In two of these, four animals dying within forty-eight hours, nothing more than congestion and hæmorrhage was made out. In the sections from seven animals, however, glanders lesions of varying size were seen. The larger foci, under a lower power, showed very numerous red-staining granules and fragments in an indefinite ground substance. Under a high power, in these foci were seen in great numbers the deeply red-stained bodies, of variable size and shape, which were observed in the lesions in other organs. Polynuclear leucocytes, structures which resembled the skeletons of nuclei and of cells, and an indefinite stroma of more or less fibrillar appearance made up the remainder of the material filling up the area. The presence of fibrin could not be demonstrated. What were considered to be beginning glanders foci were seen in the lymph follicles. These appeared as small areas in the follicles, where the nuclei were faintly stained, and in which a few deeply staining globules of varying size could be seen. In what was apparently a somewhat later stage, many of the cells of the follicles in such an area seemed to have broken up and the deeply stained globules were more numerous, while here and there a faded-out nucleus was observed. Some of the deeply stained bodies seemed to arise from changes in the nuclei of the tissue cells, for around some of them a certain amount of cytoplasm could be

seen. Sections hardened in Flemming's solution showed no evidence of fat in the foci. The process of formation of the glanders lesions in the lymphatic glands is apparently one of degeneration and necrosis with the later invasion of leucocytes, and is practically the same as occurs in other organs. The lesions do not seem to be the result of a primary proliferation of tissue cells, but are necrotic from the beginning.

*Adrenals.*—A more or less marked hæmorrhagic condition was frequently observed in these organs. In three of the nine adrenal glands examined microscopically small irregular areas were observed in which the essential condition seemed to be the degeneration of the cells of the organ, with a subsequent invasion by polynuclear leucocytes. In these areas the same deeply stained granules and bodies of various shapes were again present, in addition to the polynuclear leucocytes. A deeper staining of the nuclei and cytoplasm of the large cells of the organ was also observed in some instances. This was apparently the same change as that met with in the liver cells.

*Peritonæum.*—After intraperitoneal inoculation, the peritonæum, both visceral and parietal, may present a fibrinous exudation with minute nodules or granulations, while the omentum may be shrivelled up and infiltrated with exudation. Sections were obtained through such granulations or nodules, as well as from the infiltrated omentum. Under a low power (Plate XX, Fig. 2) the granulations appear as rounded or oval aggregations of deeply stained granules in the midst of an indefinite material, the whole being held together and limited by fibrin. Under a high power the material making up the greater part of the nodule is seen to be of two kinds—viz., the same deeply red-stained granules and irregular bodies present in the lesions elsewhere and ringlike structures not stained red. The latter are usually round, but are often oval or irregular. They vary in diameter from that of a minute globule of chromatin to that of a large cell. These structures are very numerous, and seem to represent faded nuclei of polynuclear leucocytes and other cells, as well as the cell membranes of dead cells. They were observed in the lesions elsewhere, but in smaller numbers. The fibrin is disposed mostly about the periphery

and over the surface of the peritonæum in a more or less definite layer, little or none being found in the interior of the nodule. Nuclei of polynuclear leucocytes were only rarely made out in the peritoneal nodules. The omentum on section shows larger and smaller foci and fibrinous exudation. The material forming the foci was the same as that in the nodules in the peritonæum elsewhere.

*Testicles.*—The lesions in the testicles were of the same character as those observed in other situations. The process seemed to begin usually in the tunica vaginalis with the formation of nodules of the same kind as those observed on the peritonæum. The elements making up these nodules are shown in Plate XX, Fig. 3. From the tunica the process may extend to the testicle or toward the skin of the scrotum, and large areas may be formed made up of the same material as that found in the foci elsewhere. The lesions are here again distinctly suppurative from the beginning.

*Kidneys.*—In the sections of the kidney from eight of the inoculated guinea-pigs no glanders foci were seen. These organs, however, in some cases presented marked cloudy swelling and hyaline degeneration of the epithelium of the tubules. In addition to these lesions of the epithelium, a peculiar material was frequently seen filling the lumina of the tubules, and could also be demonstrated in the capsular space of the glomeruli, apparently the same as that seen in similar situations in the human kidney. This material in some places was of a granular character, with a tendency to stain red with fuchsin; in other places it was in the form of faintly red-staining rings, with red-stained adherent granules (Plate XX, Fig. 4). The rings were of variable size, the largest being somewhat larger than the nucleus of a renal cell. They were frequently distorted or irregular in outline. The nuclei of the epithelium of the tubules were often faded and shrunken. Some were deeply and homogeneously stained, presenting the same appearance as those described in certain of the liver cells.

The origin and nature of this material in the tubules and glandular spaces is not clear. Its general appearance would suggest that it is made up of degenerated and extruded nuclei of the renal epithelium, but there does not seem to be sufficient diminution in the num-

ber of these nuclei to give support to this idea. That it represents degenerated or altered red blood-corpuscles does not seem probable, for the preparations hardened in Zenker's fluid, which is a good preservative for blood, showed no convincing evidence of such an origin.

## Conclusions.

The glanders nodule in the class of cases studied by us is in no sense analogous to the miliary tubercle in its histogenesis, and our studies afford no support to Baumgarten's views. The primary effect of the bacillus of glanders on a tissue we found to be not a production of epithelioid cells, which undergo necrosis and invasion by leucocytes, as happens in the cases in which the bacillus of tuberculosis is concerned, but to be the production of primary necrosis of the tissue, followed by inflammatory exudation, often of a suppurative character. Degenerative changes rapidly ensue in the inflammatory products. These conclusions are in harmony with the observations of Tedeschi, above referred to.

The writer desires to acknowledge his obligations to Prof. W. T. Councilman and to Dr. F. B. Mallory for many valuable suggestions in the course of this work.

## Description of Plates XIX and XX.

Fig. 1.—Small glanders tubercle in the liver of a guinea-pig, dead twenty-one days after subcutaneous inoculation. High power. The chromatin bodies, leucocytes, etc., are shown. In the periphery are seen several of the deeply staining nuclei of liver cells. Stained with fuchsin and aurantia.

Fig. 2.—Two nodules in peritonæum of a guinea-pig, dead forty-eight hours after intraperitoneal inoculation. Low power. Stained with fuchsin and aurantia.

Fig. 3.—Showing composition of the material making up a large nodule in the tunica vaginalis of the testicle of a guinea-pig, dead forty-eight hours after inoculation into the peritoneal cavity. High power. The chromatin bodies and what were considered to be skeletons of dead cells and nuclei are shown. Stained with fuchsin and aurantia.

Fig. 4.—Tubule in cortex of kidney of guinea-pig, killed sixteen days after subcutaneous inoculation. High power. Showing the peculiar structures in the lumen and the appearance of the nuclei of the renal epithelium. Stained with fuchsin and aurantia.

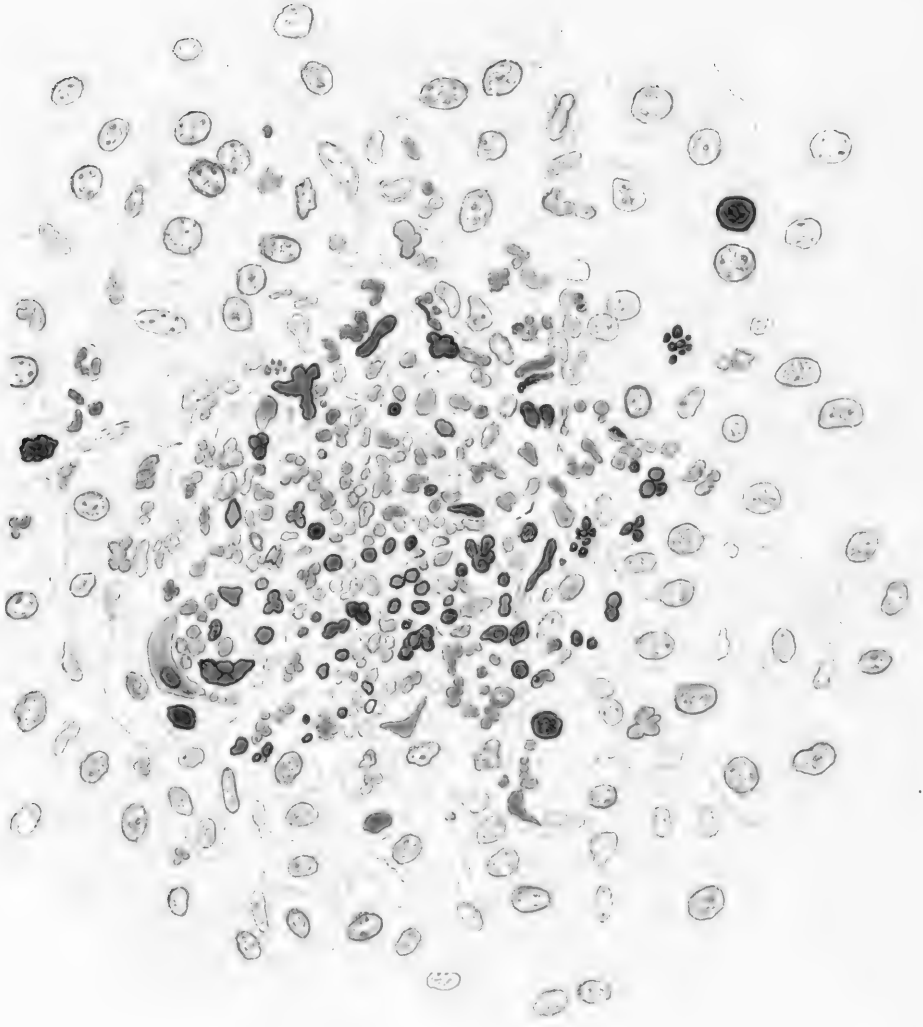

FIG. 1.

FIG. 2.

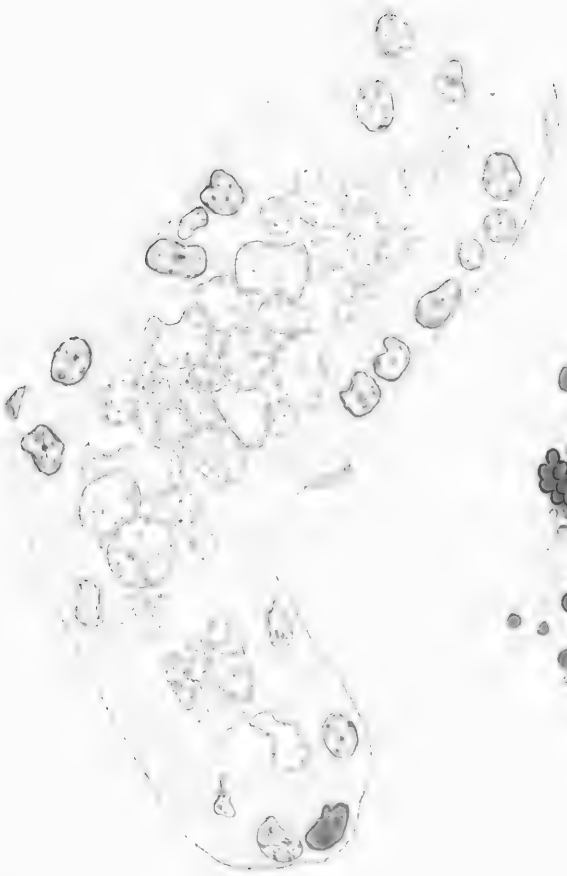

FIG. 3.

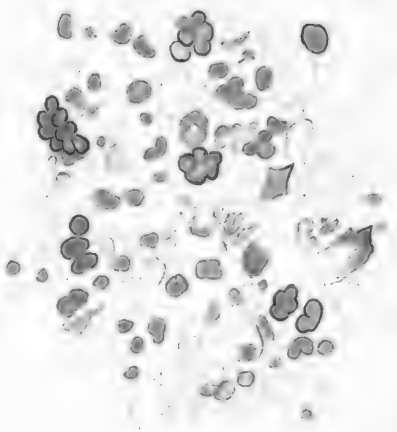

FIG. 4.

# TWO CASES OF SARCOMATOSIS WITH PURPURA HÆMORRHAGICA.

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PLATES XXI, XXII, AND XXIII.

CASES of sarcoma in which purpura hæmorrhagica has been present as a concurrent condition are comparatively rare. The volumes at our disposal contain references on the subject only by Hilton Fagge* and Railton.† According to the latter author, the subject has also been studied by Kingsford and Anderson, though we could obtain no evidence of their work in the literature.

Fagge, in an interesting article published more than fifteen years ago, gives six records taken from the *Guy's Hospital Reports*. In these cases purpura and sarcomatous tumours were coincident, so far as macroscopic appearances and clinical observations could verify the conditions. In four of the cases, however, no microscopic examination was made, while in the two remaining instances the pathological reports and microscopic examinations are, to say the least, unsatisfactory.

The most interesting of all these is perhaps Case I, in which the purpura was present about the chest and abdomen, the hæmorrhagic infiltration appearing in the form of narrow rings surrounding round, elevated, hard nodules, the conditions being evidently identical with those found in our second case. There were also the same tendency of the cells to infiltrate the adipose tissue and the same visceral hæmorrhages. The main growth, which began in the ileum, was made up of small round cells.

*Hilton Fagge, *Guy's Hospital Reports*, vol. xxv.
† Railton, *British Medical Journal*, May 18, 1895.

In three of the other cases there were no nodules in the skin.

In one instance in which no autopsy was obtained the multiple growths in the skin and glands appeared as flattened thickenings in the brownish-stained skin. The purpura seems to have formed chiefly around the hair follicles. In the last case of all, where no microscopic examination was made, the skin presented many swellings, which on section seemed to contain merely fibrin and blood.

Out of all six cases, then, a microscopic examination of the skin was made in but one instance, and even in this no report of the condition of the vessels is recorded. In his concluding remarks the author suggests the possibility that the prevalence of purpura having been first noted in these cases, the hospital records of instances of so-called Werlhofs's disease may in reality refer to conditions of sarcomatous origin, the primary growth, perhaps, remaining obscured in the medulla of the bones or elsewhere. From the fact, however, that in three of his cases the onset was ushered in with a chill, and that there had been a previous exposure to wet, Fagge regards this factor as a possible direct cause of sarcoma, in which case the multiple tumours would have to be considered not as metastases, but rather as a uniform development of numerous growths, each in itself primary.

Fagge advances several possible explanations of the relationship between sarcoma and purpura. The first, as has been already indicated, supposes that a minute development of sarcomatous tissue, with vessels made up of embryonic cells, takes place at each effused spot, apart from any infection or appearance of metastases, and that the conditions are somewhat analogous to those which occur in molluscum fibrosum. The second explanation is based upon the embolic theory, the sarcoma cells reaching the capillary vessels. Thirdly, Fagge deems it not impossible that the condition of cachexia, with its altered blood state, which is of frequent occurrence in sarcoma, might of itself suffice to produce the purpuric condition, the sequence of events being analogous to that which characterizes the grave anæmias.

In the case recorded by Railton the skin of the head was mainly

affected, large sarcomatous nodules existing in this region, with hæmorrhages in their immediate vicinity. There were elsewhere no cutaneous effusions. The liver and retroperitoneal glands were the only organs showing sarcomatous infiltration.

The cases which have come under our notice are as follows:

Case I.—Janet H., aged thirty-five years, was admitted to Prof. James Stewart's wards in the Royal Victoria Hospital on May 6, 1895, complaining of anorexia, great thirst, which had existed for one week previously, with pain in the epigastrium, increased by pressure. For the past two months she had been losing flesh. Two days prior to admission she observed a discoloured patch on the right forearm about the size of a twenty-five-cent piece, and had also some bleeding from the gums. On the following day she vomited a cupful of dark-coloured bloody fluid; the bleeding from the gums continued, and further areas of subcutaneous hæmorrhage appeared; these rapidly increased in size and number up to the day of admission.

She was a Canadian, with previous good health, and accustomed to good diet. There was a family history of tuberculosis. Menstruation had always been regular and unaccompanied with pain.

*Condition on Admission.*—The patient was stout and powerfully built, although rather anæmic; the gums were somewhat swollen and hæmorrhagic; the tongue was coated and fissured, and showed hæmorrhages upon the dorsum. Numerous subcutaneous hæmorrhages of various sizes existed over all parts of the body. Some were bright red, others brownish; whereas many were diffuse, extensive, irregular, and not elevated, others were smaller, firmer, distinctly nodular on deep pressure, and painful. There was no evidence of glandular enlargement.

The teeth were loosened in the alveolar processes, with blood oozing about them. The breath was foul, the appetite poor, and there was great thirst. No evidence of abdominal pain or tenderness. The liver and spleen were apparently normal in size. The urine was very acid (specific gravity 1017), and contained some albumin, a few casts, and a heavy deposit of amorphous urates and uric-acid crystals.

Blood examination showed 3,300,000 red corpuscles; moderate leucocytosis, with forty-five per cent of hæmoglobin.

In stained preparations there were present a few eosinophiles and free eosinophilous granules.

The subsequent history was that of rapid failure up to death, which occurred within ten days. During this period the patient vomited sev-

eral times, losing in all more than three pints of a grumous bloody fluid. An ascaris lumbricoides was present in one vomitus.

The diagnosis was reserved; scurvy was suspected.

Autopsy (Performed three hours and a half after death; notes by Prof. Adami).—The body was that of a stout, broadly built adult woman, 186 centimetres long, nearly 50 centimetres across the hips. The face approached the negro type, and the complexion was swarthy. Gums anæmic, slightly swollen around the bases of the teeth, and presenting here a fine line of exuded blood. The upper and lower incisors and bicuspids loose. A small, deep hæmorrhage in the lobe of the left ear. Numerous punctiform petechiæ upon the neck. On the arms the skin was discoloured in patches, some areas measuring as much as 10.5 centimetres in diameter. These patches were irregularly distributed over the arms, but showed no elevations whatever. In both axillary regions there were clusters of petechiæ. On the trunk there were very many punctate hæmorrhages, forming a zone around the body above the umbilicus. There were no signs of hæmorrhage in the exposed mucous membranes of the vagina. The legs were affected similarly to the arms. In some places the hæmorrhages existed definitely at the roots of the hairs, while in other places there seemed to be no relation between the purpuric spots and the hair follicles.

*Abdominal Cavity.*—Massive panniculus, nearly 8 centimetres thick; very numerous petechiæ in the parietal peritonæum. The omentum large and fatty, and presenting numerous small hæmorrhages in its substance. The serous coat of the intestine and the appendices epiploicæ showed multiple punctate ecchymoses. There were about 20 cubic centimetres of turbid, faintly blood-stained fluid in the abdominal cavity.

*Spleen.*—Very large and heavy, measuring $18.3 \times 11 \times 4$ centimetres, and weighing 630 grammes. The edges were rounded and without notches. It was firm to the feel, and the surface presented a coarsely mottled appearance, with distinct yellowish areas not projecting from the deep red groundwork of splenic substance. These areas varied in size up to that of a split pea, and were not well

circumscribed. On section the organ was very firm, presenting throughout its substance the same yellowish areas of new tissue. Hilum normal.

*Kidneys.*—The right kidney slightly enlarged; the easily peeled-off capsule revealed a pale-yellow smooth surface, upon which could be seen a few small grayish-yellow nodules, resembling those in the spleen. On section it presented evidence of much fatty change, and a few minute new growths were distributed throughout the cortex. The pelvis and ureters were normal. The left kidney resembled the right.

*Bladder.*—Mucosa pale; upon it were numerous rounded, slightly projecting masses about 1 millimetre in diameter, around which there were punctate hæmorrhages.

*Vagina.*—Normal and without ecchymoses.

*Uterus and Adnexa.*—Enlarged; the wall greatly thickened and for the most part firm, though posteriorly there could be seen softer areas. The cervical portion especially enlarged, owing to the presence of a new growth, which appeared as a large rounded infiltrating mass, chiefly on the posterior portion. The mass was not sharply limited on all sides, but was continuous with tissue of a similar character in the body of the uterus itself. There were a few minute cysts centrally situated. The growth measured about 8 or 9 centimetres in its greatest diameter, and projected backward and somewhat upward, so that its superior part lay behind the body of the uterus itself. It was fairly firm on section, and free from juice. From the posterior portion of the fundus there was given off a firm pedunculated, subserous fibroid tumour, slightly lobulated, the greatest diameter of which measured 3.5 centimetres.

The right Fallopian tube was slightly enlarged, and was partially bound down by a few adhesions to the surrounding tissues. The fimbriated extremity was free. The right ovary was much enlarged, and contained a cyst 6 centimetres in diameter, with delicate transparent walls and thin, greenish mucoid contents. The solid tissue remaining was dense and uniform, measuring about 2.5 centimetres in thickness. The left tube was tortuous from old adhesions and en-

larged; the fimbriated extremity was free. The left ovary was greatly enlarged, partly from new tissue formation, partly owing to the presence of several cysts. It was fairly firm on section. The new growth seemed to extend upward, infiltrating the tissues of the broad ligaments and also of the Fallopian tubes. On the peritoneal surfaces of both ovaries there were numerous ecchymoses.

*Stomach.*—Large, with moderately thin walls and coffee-ground contents. Along the greater curvature at the middle of the cardiac convexity was a circular, flat, soft, grayish tumour 2 centimetres in diameter, projecting into the stomach cavity. The mucosa over it was superficially ulcerated; the edges thick and rounded. About the middle of the greater curvature there was a similar growth, and another 2 centimetres in diameter on the posterior wall, somewhat nearer the pylorus. In addition to these there were several small whitish nodules, which rendered the mucosa firmer and thicker than normal, and in one of them there were small hæmorrhages. There were no external evidences of new growth.

*Intestine.*—Throughout the small intestine there were minute submucous petechiæ; otherwise no abnormality existed. The large intestine was normal, save for a few ecchymoses near the sigmoid flexure.

*Liver.*—Some fatty infiltration; lobules distinct; no evidence of hæmorrhage.

*Mesenteric Glands.*—Normal.

*Thoracic Organs.*—In the mediastinal tissue there were a few minute ecchymoses. The pleural cavities were dry and free from adhesions. Lungs normal.

The heart showed ecchymoses on both layers of the pericardium and a patent foramen ovale; otherwise no abnormality.

Examination of the brain was not permitted.

*Cultures* on glycerin agar and on broth from the subcutaneous hæmorrhages, spleen, liver, heart's blood, and kidneys all remained sterile.

Microscopic Examination.—The *spleen* showed great congestion, very marked leucocytosis, much overgrowth of the lymphoid elements, and large numbers of small round cells lining the interior of

the sinuses. It is especially to be noted that wherever these cells were found their inner boundary was always limited by a single layer of spindle cells, resembling the intima of a vessel. (Plate XXI, Figs. 1 and 2.) In some places the trabeculæ showed a distinctly abnormal number of small round cells following their direction. The Malpighian bodies were poorly outlined. The arteries were apparently free, and showed no evidence of emboli.

The *kidneys* showed, in addition to some parenchymatous degeneration, localized areas of small-celled infiltration, chiefly in the periphery of the organ. The new cells were most numerous between the tubules, often crowding them to one side and obliterating the lumina. The glomeruli in the neighbourhood were likewise surrounded by small round cells, their capsular epithelium was frequently proliferated, and their afferent blood vessels in some places seemed to be surrounded by small round cells. In most cases the greatest cellular infiltration was in the immediate neighbourhood of the glomeruli.

Sections of the *cervix uteri* presented the usual appearances of an infiltrating, small-round-celled sarcoma. There were a few punctiform hæmorrhages. The blood vessels presented free lumina, although there was infiltration both of the coats of many of the vessels and of the tissues immediately about them.

The *ovary* showed similar sarcomatous infiltration, there being but little of the normal tissue visible.

Sections through the tumour masses of the *stomach* showed a large amount of catarrhal exudate on the mucosa, extensive small-celled infiltration and new formation both between the gland structures themselves and in the submucosa; there was but slight infiltration of the muscular coat. The blood in the vessels showed considerable leucocytosis. The lumina of the vessels themselves were quite free from emboli in the sections examined, but the perivascular lymphatics showed very great infiltration with small round cells. In some places the vessel walls themselves were infiltrated with small round cells, though nowhere to a marked extent. There was a moderate amount of recent hæmorrhage in the submucosa.

The *liver* showed generalized infiltration of small round cells along the portal sheath. The growth had here extended into the wall of the vessel, and was bounded only by a thin layer of endothelial cells. There were no evidences of emboli within the vessels. The liver otherwise was moderately fatty, and the cells contained much bright-yellow pigment. (Plate XXII, Figs. 3 and 4.)

Sections of the *heart* showed over the hæmorrhagic areas of the pericardium some small collections of round cells, old and recent extravasations of blood, but no other pathological conditions.

The *skin* presented circumscribed nodules of small round cells, a great infiltration of the adipose tissue, and an involvement of the arteries and their coats similar to that described as present in other organs.

The *adrenal bodies* showed about the central vessel a slight amount of small-celled infiltration, but were otherwise normal.

In the *omental glands* there was an extensive growth of small round cells, corresponding in character to those above described.

Anatomical Diagnosis.—Sarcoma of cervix uteri and of ovaries, involving also the stomach, spleen, kidneys, adrenal bodies, liver, and skin. Purpura hæmorrhagica of skin, mucous membranes, and viscera.

Examination, therefore, of the tissues in this instance shows the sarcoma to have spread by various channels—viz., the lymphatics, especially those about the blood vessels, and further by an infiltration of the walls of vessels, which seemed to dissect out between the intima and muscular as well as the adventitial coats. There was no evidence of thrombosis in the veins or capillaries.

Emboli were nowhere found, although the portal venous system showed abundant evidence of the presence of sarcomatous growths.

Case II.—Mary R., aged twenty-eight, entered the Royal Victoria Hospital on July 4, 1895, complaining of spitting blood and of the presence of bluish spots upon the limbs and body. The expectoration of blood had begun three days previously, and had been more or less continuous. There was also some hæmatemesis at the onset. The symptoms were not attended with cough. The dark spots on the limbs and

body were first observed five days prior to admission; they appeared first at the outer side of the right knee, and were accompanied with pain and tenderness; the colour, at first reddish, changed to pale brown, and then to darker brown, and the tenderness ceased. Rapidly following on this first effusion numerous other patches of various sizes appeared on the lower extremities, arms, and trunk.

Her past history was one of gastric disturbance, with frequent vomiting and some constipation during the past five years. There was no history of previous hæmatemesis, and no other evidence of gastric ulcer. She was a Parisian and unmarried. No history of deprivation or hardship. Her occupation was that of a dressmaker. The family history was tuberculous. There was no history of hæmophilia.

*On admission* she was weak, intensely pale, and was bleeding from the gums and other mucous membranes, while in the skin there were numerous subcutaneous hæmorrhages. Of these, some were slightly elevated, with a smooth surface and well-defined edges; in size they ranged from a five- to a ten-cent piece, some being circular, others crescentic. There were, however, some elevations of a similar size with hæmorrhages. Other large, diffuse, irregular subcutaneous hæmorrhages were not raised, and existed mainly in the folds of the buttocks and the inner surface of the left thigh; there were also many smaller non-elevated patches distributed over the body. The abdomen presented, a day or two after admission, a few areas as large as twenty-five-cent pieces, distinctly raised, and not uniform in colour. In the periphery of these there was a definite narrow ring of hæmorrhage, resembling very much the marks of an aniline pencil, while within the circle was a pale centre. Temperature, 99° F.; pulse, 120; respirations, 30. There was elsewhere no evidence of disease, but a vaginal examination was omitted.

During the patient's sojourn in the hospital the hæmorrhages increased, and her general condition became worse, the temperature higher, rising to 103° F., and the pulse ranging from 122 to 160. The respirations progressively increased up to 60. Some of the raised spots became flatter and faded, while others remained stationary. The urine and fæces were normal. The blood examination showed the red cells to vary between 1,000,000 and 2,000,000, the white cells 50,000, with a special increase in the lymphocytes. The hæmoglobin was about twenty-five per cent.

The treatment consisted in the administration of liq. arsenicalis, and later liq. calcis chloridi, in twenty-minim doses three times daily. The treatment otherwise was purely symptomatic, and, on the whole, was of no avail, death ensuing rapidly.

The diagnosis was multiple sarcomata with purpura hæmorrhagica.

Autopsy.—The body was that of a poorly nourished woman of about thirty years of age. Rigor mortis present; lividity in dependent parts. Mammæ rather poorly developed; the right moderately soft, the left presenting a small, firm movable tumour, well circumscribed, and not involving the skin. On the abdomen and chest were six or eight areas of bluish discoloration, more or less circular in outline, the largest equal in size to a twenty-five-cent piece. Their boundaries were remarkably well defined, and they had a firmer consistence than the surrounding skin. On section, also, they seemed firm, presenting free blood and blood pigment in the tissues. On the buttocks and at various parts of the lower extremities were several much larger effusions of blood beneath the skin, the largest about 12 centimetres in diameter and of very irregular outline. These latter, however, did not present the same firmness as that manifest in those on the abdomen. The visible mucosæ pale. No œdema.

*Abdominal Cavity.*—Panniculus adiposus moderately developed, with a yellow atrophic appearance; muscles brownish red in colour. The pelvis contained a small quantity of thin reddish fluid; the liver not visible below the ribs; the intestinal coils distended, their serosæ smooth and glistening, presenting here and there patches of hæmorrhage beneath. In the pelvic cavity were two large irregularly oval tumours, evidently connected with the adnexa of the uterus, freely movable with the tubes, and apparently ovarian (see below).

*Spleen* measured $14 \times 10 \times 4$ centimetres; weight, 340 grammes; large, smooth, of a uniform bluish-red colour, fairly soft on section, with apparent increase of pulp. Malpighian bodies indistinct.

*The kidneys* gave evidence of an acute nephritis, with engorgement of the medullary vessels. Beneath the mucosa of the pelvis and some of the calices of the left kidney there was some diffuse recent hæmorrhage. In the right kidney there was moderate hydronephrosis, some thickening of the ureter and narrowing of its lumen halfway down to the bladder.

*The bladder* showed a pale mucosa, with two or three punctiform hæmorrhages beneath it.

*The liver* was small and somewhat friable; its colour brownish.

*The uterus* was of about normal size; walls firm, especially at the cervix. Tubes apparently normal.

*The ovaries* were both converted into large more or less solid tumours; the left, which was reniform, measured $9 \times 5 \times 4$ centimetres; it was in the main solid, though fluctuating in parts. The surface, though somewhat nodular, was smooth and glistening, and presented some subperitoneal hæmorrhages. On section the solid part was homogeneous, free from juices, and contained a few minute hæmorrhages. The fluctuating mass contained a clear, thin fluid. The right mass was slightly more rounded and larger; it weighed 60 grammes. It was more fluctuating than the left, owing to the presence of smooth-walled cysts and cavities containing hæmorrhagic detritus, as well as other degenerated material. The vessels in the neighbourhood appeared free, and there was elsewhere no evidence of abnormality in the pelvic organs.

*Thoracic Cavity.*—In both pleural cavities there were about 100 cubic centimetres of a thin, turbid, reddish-brown fluid. On removal of the sternum, the lungs retracted in part only, being held fixed by loose adhesions at apex and base and by firmer adhesions in the central portions. The left lung showed, in addition to moderate œdema and anthracosis, some minute recent hæmorrhages into its substance; the larger blood vessels were free, the bronchi reddened. The right lung was similar in general character to the left, the hæmorrhages being likewise minute, and distributed more at the base than at the apex.

*The Heart.*—The pericardial cavity contained about 30 cubic centimetres of clear yellow serum. The surface was smooth and glistening. Beneath the visceral pericardium and over different areas of the heart there were numerous hæmorrhagic patches of a bright-red colour, none of them appearing old or of long duration; these were distributed most markedly over the left ventricle, almost covering its entire surface. In the various chambers of the heart there was a small quantity of blood. The various orifices and valves were normal. Beneath the endocardium of both the right and left sides of the heart there were likewise hæmorrhages of small size, and within

the heart muscle interstitial ecchymoses. There was early atheroma of the aorta. The peribronchial glands were normal.

*The brain* was free from hæmorrhages, and showed no abnormality beyond some congestion.

*The thyroid gland* showed bilateral enlargement with colloid degeneration, but no hæmorrhages. The remaining organs of the neck were normal.

*Cultures* on glycerin agar from the heart's blood, spleen, and liver remained sterile.

Microscopic Examination.—*The ovarian tumours* presented the characteristics of small-spindle-celled sarcomata, with a generous admixture of small round cells. There were large, irregular patches of hæmorrhage and great engorgement of the veins. A considerable amount of myxomatous change had supervened, while in other parts a kind of hyaline degeneration was apparent. The blood vessels, and especially the arteries, were in many cases seen to be packed with small spindle cells, even in directly transverse sections of the vessels.

*The lymphatic glands* in the region of the stomach and pancreas had but little of their lymphoid structure left, the specimens showing great infiltration, with small spindle cells arranged in the manner usual in sarcomatous growths.

*The spleen* showed great congestion; the Malpighian bodies were not very distinct, and the fibrous tissue was about normal in amount. There was no sign of new growth, nor evidence of hæmorrhage.

*The kidneys* were congested; the glomeruli apparently normal; the convoluted tubules in some cases showed cloudy swelling, in others advanced degeneration. In the sections cut there was no evidence of hæmorrhage.

*The Stomach.*—Sections cut through the hæmorrhagic portion showed both old and recent pigment. In the neighbourhood of some of the hæmorrhages there was distinct evidence of necrosis in the mucosa, submucosa, and muscular coat. There was, in addition, a small amount of cellular infiltration, with great congestion of the submucosa. The vessels of the stomach, and especially the arteries, showed in many places the lumina filled with spindle cells. In some

of them, cut transversely, blood-corpuscles and numerous spindle cells were irregularly intermingled. From the large number of these cells, and from the fact that they were massed together to such an extent as in many cases to occlude completely the lumen, it is hardly likely that they were endothelial cells; and, moreover, their outlines presented the characters of young connective-tissue cells rather than those of endothelial cells. (Plate XXIII, Figs. 5 and 6.)

*The small intestine* showed great congestion and hæmorrhages of moderate degree, with superficial necrosis of the mucosa; in addition, a considerable amount of small round-celled infiltration was apparent between the glandular structures.

*The liver* showed brown atrophy of the cells, but no evidence of hæmorrhages. In certain irregular areas in the periphery of the lobule there was a moderate amount of small-round-celled infiltration. None of the vessels contained emboli.

*Heart Muscle.*—In the subpericardial fat there was a large amount of recently extravasated blood, and there was also very much cellular infiltration, the cells infiltrating the tissues between the fat globules and being closely packed together. In many places the hæmorrhage and the cellular infiltration existed side by side. Within the heart substance there were many patches of recent hæmorrhage, with slight necrosis of the muscle fibres. In the vessels there were many spindle cells, while outside the adventitia of many arteries there were fairly large collections of small spindle cells closely packed together.

*The lungs* showed in some parts thin septa, whereas in other portions the meshes of tissue composing the septa were widely separated, apparently from œdema. There was but little cellular exudate in the alveoli, but here and there many patches of hæmorrhage were apparent. The blood vessels in the neighbourhood did not present very much abnormality, though in some of the arteries could be seen collections of small spindle cells, the signs here being apparently merely those of a desquamative angeiitis.

*The Skin.*—Sections were taken not only from parts distinctly nodular in appearance, but also from the more diffuse hæmorrhages. In the nodular hæmorrhagic portions the epidermis and papillæ were

fairly well marked in some places, while in others there was distinct flattening of the papillæ. The subcutaneous tissue seemed thickened, the meshes being in some portions widely separated, as though from œdema. The sudoriparous glands and hair follicles presented no abnormality, there being but a small amount of cellular infiltration in their vicinity. The hæmorrhages were for the most part deeply situated in the subcutaneous tissue. Many of the vessels in the neighbourhood were plugged with spindle cells, though in the vicinity of these there was no sign of sarcomatous growth, and the tissues stained poorly, being necrosed.

The conditions present in the latter case, therefore, show primary sarcoma of the pelvic organs, which had, to some extent at all events, advanced by way of the lymphatic system, and involved the glands in the neighbourhood of the cœliac axis, as is apparent both from the macroscopic and microscopic examinations. A more minute examination of the primary seat showed involvement of the vessels in the tumour, spindle cells filling their lumina. In the other tissues, moreover, and especially in those parts where hæmorrhage existed, the perivascular lymphatics appear to have conveyed the cellular elements, for on examination of some of the various affected tissues there were found in the perivascular spaces accumulations of spindle cells, with a moderate amount of hæmorrhage in their immediate vicinity.

## General Conclusions.

The purpura accompanying the two foregoing cases of sarcomatosis would seem to find its explanation in the coexistence of several factors, the main feature being an involvement of the vascular system by the sarcomatous elements.

There existed in Case I a direct lesion of the vessel wall whereby the sarcoma cells invaded directly the various coats, and were found mainly between the intima and the adventitia, dissecting their way, as it were, along these tracts in the vessel walls. There was further an extensive involvement of the perivascular lymphatics, from which point, indeed, it would seem that the sarcoma cells had invaded the walls of the vessels themselves.

In Case II, moreover, not only was there a definite invasion of the lymph spaces near the vessels, but, furthermore, there was undoubted evidence of the existence of emboli of sarcoma cells in the lumina of the blood vessels; and in the immediate vicinity of such conditions hæmorrhages were invariably found. While some vessels, and indeed a great many, were quite free from such emboli, in others the lumina were completely occluded by spindle cells, so as to preclude the possibility that these were merely a collection of desquamated endothelial cells, such as is frequently found as the result of post-mortem changes. That such an embolic condition can exist is by no means an unreasonable supposition, and, while it is generally recognised that multiple sarcomata are usually made up of small round cells, in this case we have an undoubted example of sarcomatosis of the spindle-celled variety. There are numerous instances of this "embolic purpura," as it may be called, especially in French and German literature, the condition being associated with rheumatism, valvular lesions of the heart, and other diseases which induce directly or indirectly the formation of emboli. Krauss,* Gimard,† Leloir,‡ and others have insisted with considerable emphasis on the embolic origin of many purpuric conditions, and in some instances they have verified their observations by histological examination.

Leloir assumes that, in addition to the presence of the ordinary emboli and the changes in the vessel walls with desquamative endarteritis, the blood itself may be much altered chemically, and that in the cachectic conditions clots may be thrown down from the circulating blood and be carried onward to form capillary emboli, with resulting hæmorrhagic infarctions.

Krogerer,# some ten years ago, in examining the skin removed from patients with symptomatic purpura, found definite thromboses in the smaller veins, and even in the arteries. According to his view, the alterations in the vessel walls gave rise to slowed circulation and tendency to thrombosis, bringing about a liability to hæmorrhages.

* E. Krauss, Ueber Purpura, Heidelberg, 1883.

† Gimard, *Arch. gén. de. méd.*, October, 1883.

‡ Leloir, *Annales de dermatologie et de syphiligraphie*, 2. sér., t. v, p. 1, 1884.

# Krogerer, *Zeitschrift für klin. Medicin*, 1885, Bd. x, Hft. 3.

His plates bear out his theories regarding the thrombi, many of which show considerable organization.

But a careful examination of the purpuric areas shows further that a mere invasion of the vascular system by sarcoma cells can not explain all the various blood effusions present. On examining the skin, for instance, in those areas where large irregular hæmorrhages had occurred, there was but little evidence of vascular invasion, while the emboli, on the other hand, seemed to exist mainly in the localized smaller and more circumscribed patches. One must therefore conclude that in such instances a combination of factors will alone afford a rational explanation of the purpura, and that in the general condition of the patient we shall find another cause for the enormous effusions of blood. In both of our cases there were high fever, cachexia, and a rapid progressive asthenia, all being the results of a sarcomatosis, and implying also grave alterations in the composition of the blood. From this we may infer an altered condition of the vessel walls, and hence probably a combination of circumstances sufficient to explain the incidence of hæmorrhage.

The raised cutaneous nodules in our second case, some of which were hæmorrhagic, can not be regarded as pure sarcomatous metastases, for on microscopic examination they merely revealed hæmorrhage or necrosis, or both, and sometimes plugging of the vessels. There was nowhere in these nodules evidence of new growths. Such elevations, then, must have been produced rather by a temporary serous or cellular exudation coincident with or following upon the hæmorrhage—a probability which is emphasized by the fact that during the last days of the patient's illness many of the nodules diminished in size. Whether the œdema and infiltration were secondary to the embolic process in the subcutaneous vessels or whether they were merely coincident with the hæmorrhage would be difficult to decide.

The ringlike spots, however, are of special interest, inasmuch as it has been shown that they have been present in more than one case of sarcoma. It is not impossible that such spots may be definitely related either to the embolic processes or to a direct invasion of the

cutaneous vessels, though, so far as we know, there do not exist any experimental proofs to bear out such a theory. From what has been said, however, it is evident that the cutaneous vessels were plugged during the last few days of the illness, at a time when the walls of the smaller vessels and capillaries were already greatly enfeebled. The result of the embolic formation may therefore mean a decided deficiency in the supply of nutriment to the involved area, the collateral circulation naturally being poor under the circumstances. As soon, then, as the vessels had become plugged, the surrounding blood supply would be poured in to a limited extent, and, on meeting the enfeebled vessels, might possibly break through their thin walls, thus producing a zone of hæmorrhage around the area deprived of its normal nutrition. In other words, the condition may be regarded as in many respects analogous to that presented in embolic infarcts in regions with end arteries, central necrosis with peripheral congestion and hæmorrhage being induced, the latter being chiefly limited to the outer zone of the necrotic area. The cutaneous vessels under such circumstances may be regarded as end arteries in a functional sense, since the collateral circulation would be so diminished under the altered conditions that no complete nourishment could be afforded to the area supplied normally by the plugged vessel. Von Recklinghausen * has directed especial attention to the occurrence of cutaneous hæmorrhages following embolic or thrombotic occlusion of peripheral arteries.

The possibility of some toxic condition as a factor in the production of the purpura in our cases may also be suggested; but while we would not exclude this possibility, we are unable to find any positive evidence in its favour. Focal necroses, which are often associated with toxic and infectious processes, were present only in direct association with the hæmorrhages, and were not distributed in the liver, spleen, and kidneys in the manner characteristic of toxic infections. Nevertheless the absence of these necroses does not exclude the possibility of the existence of some form of toxæmia. Infection demon-

* Von Recklinghausen, Handbuch der allg. Pathologie d. Kreislaufs u. der Ernährung, p. 160, Stuttgart, 1883.

strable by bacteriological examination was absent, and there is no reason to regard our cases as allied to the infectious purpuras.

The thermic theory suggested by Fagge at all events finds no place in the production of the multiple tumours in our cases, inasmuch as in each instance extensive visceral growths had given rise to the metastases.

In conclusion, we would express our gratitude to Prof. J. Stewart for permission freely granted to record the cases, as also to Prof. Adami, the director of the laboratory, who not only gave us much assistance in the preparation of this paper, but kindly undertook the entire work connected with the appended illustrations.

## Description of Plates XXI, XXII, and XXIII.

Fig. 1.—Spleen from Case I. Low power, × 55. Masses of small round sarcoma cells are seen apparently lining the sinuses of the spleen. The other structures visible in this specimen are normal.

Fig. 2.—Part of the same under high power, × 240. This section shows the sarcoma cells on the inner margin of the splenic sinuses; they are separated from the lumen of the sinus by a thin layer of endothelial cells, E. At one portion the cells are seen infiltrating between the fibrils of the sinus wall, I. M, the main wall of the sinus; P, the spleen parenchyma; S, sarcoma cells.

Fig. 3.—Liver from Case I. Low power, × 40. The section shows a moderate small round-celled infiltration in the periphery of the hepatic lobule, following mainly the portal sheath.

Fig. 4.—Part of the same section under high power, × 240. The small round sarcoma cells are infiltrating the portal sheath, and may be seen covered by a thin layer of endothelial cells; the condition is similar to that seen in the spleen. A, H, arteria hepatica; B, D, bile duct; L, liver cells; V, P, vena portæ.

Fig. 5.—Section through the stomach wall from Case II. Low power, × 40. The arteries of the submucous coat are seen plugged with spindle cells, and in their immediate vicinity there are old and recent blood extravasations. H, hæmorrhage; N, necrosis; E, embolus of sarcoma cells.

Fig. 6.—The same under high power, × 180. B, blood-corpuscles; E, embolus of sarcoma cells; N, necrosed tissue.

FIG. 1

FIG. 2

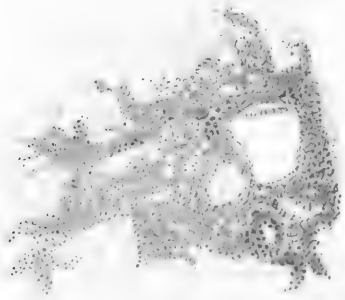


FIG. 3

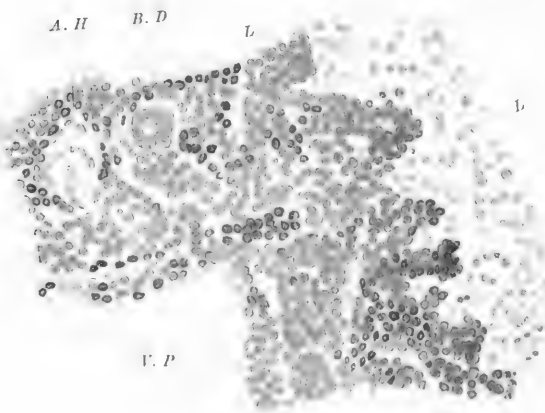



FIG. 4

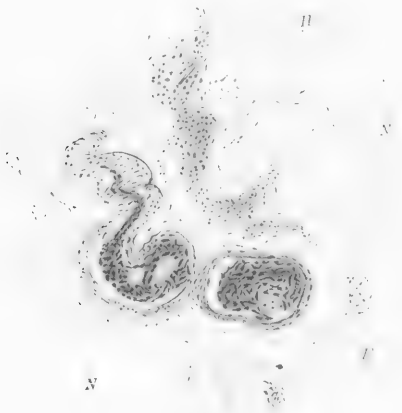
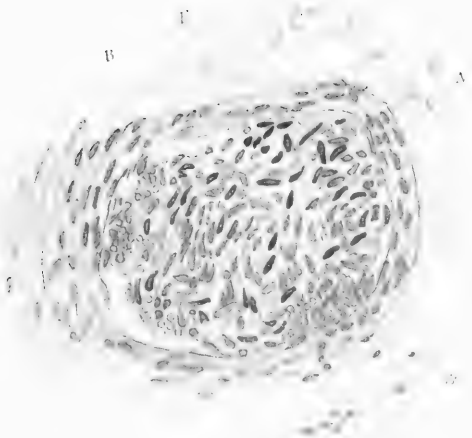



FIG. 5



# A STUDY OF THE CHANGES PRODUCED IN THE KIDNEYS BY THE TOXINS OF THE STAPHYLOCOCCUS PYOGENES AUREUS.

By JOHN LOVETT MORSE, A.M., M.D.

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PLATES XXIV AND XXV.

THE lesions which are produced in the kidneys by the staphylococcus pyogenes aureus have been carefully studied. Experiments made by injecting pure cultures of the organism into susceptible animals go to show that the resulting lesions are of the same general character as those observed in man. In the human kidney they appear as smaller or larger abscesses and foci of necrosis which have the form of infarctions. The number, situation, and size of the abscesses differ in different cases. They may be homogeneously distributed through both cortex and pyramids, and are often arranged in rows, a single abscess in depth, of which the general direction is parallel to that of the tubules. In the case of these single abscesses, especially those which appear in the pyramids, the length almost always greatly exceeds the breadth, their long diameter being at right angles to the surface of the kidney, and following the course of the collecting tubules.

The effects produced in the kidneys of rabbits by the injection of virulent cultures of this organism into the ear vein differ, however, in some respects from those seen in man. The lesions appear in the form of small white, rather firm foci, which are usually more numerous in the pyramids than in the cortex. On microscopical examination of such a focus, a mass of organisms is found in the centre. Around this there is an area of complete necrosis, in which the tubules filled with granular material can be recognised, and external to this again is seen an area of purulent infiltration.

In the human kidney there is usually more definite abscess formation, and the sharply circumscribed area of necrosis around the central mass is generally absent. In man, however, when the conditions of infection can be considered as equivalent to the injection of large numbers of organisms into the blood at one time, lesions identical with those seen in the rabbit may be produced. This fact we have ourselves verified by observations made upon cases in which after criminal abortion the infection had come through the uterine veins with or without a secondary staphylococcus endocarditis. In these cases the naked-eye lesions may be confined to the pyramids, and appear not as abscesses, but as small necrotic foci, with their long axes parallel to those of the tubules. These foci in the pyramids may have a double source of origin. Even when macroscopically lesions are not apparent except in the pyramids, a careful microscopical examination will show masses of the cocci in the blood vessels of the glomeruli, and in some cases even in the capsular spaces, whence the organisms can easily be washed down along the course of the tubules, and by their gradual growth occlude either the loops of Henle or the collecting tubules. In many cases, owing to the necrosis of the tissue, it is impossible to say whether the organisms in the pyramid are lying in a tubule or in a vessel. In some cases they appear undoubtedly to be within the lumen of a small vein; and it is possible that the organisms circulating in the blood of the kidney find in the slow circulation of the veins of the pyramid suitable opportunities for growth, and, first growing along the wall, finally completely occlude the vessel. We are inclined to believe that the organisms lie more frequently in the veins than in the tubules. The anæmic necroses found both in man and experimentally are due to the gradual occlusion of the lumina of the terminal arteries by the lodgment and growth of the organisms.

In addition to these focal changes, which result from the direct local action of the organisms and their toxins upon the tissues, there are more diffuse lesions which must be referred to the action of soluble substances. In almost all cases of staphylococcus septicæmia seen in man there is a greater or less degree of granular, fatty, and hyaline degeneration of the renal epithelium, which sometimes amounts to

complete necrosis. The same condition is seen in rabbits, and Ribbert has shown that the physiological function of the cells so altered is affected, and that they do not, after the injection of carmine into the blood, secrete this substance, as do the normal epithelial cells. The interstitial tissue of the kidney shows a degree of participation in the pathological process which varies directly with the intensity of the focal lesion; and there is always a more or less extensive new formation of cells in the interstitial tissue around the areas of purulent infiltration. Ribbert, in his experiments with injections of the staphylococcus, has found in animals which have lived for several weeks small foci of cellular infiltration in the interstitial tissue not connected with the presence of organisms. The epithelium of the tubules in these foci shows various degrees of degeneration, and Ribbert is disposed to regard the interstitial growth as secondary to the epithelial degeneration. In some of the human kidneys observed by us the same thing was noted. In these cases not only was there quite an extensive growth of the interstitial tissue extending for a considerable distance beyond the limits of the abscess, but, in addition, there were foci of interstitial cellular infiltration scattered through the kidney, and not connected with the abscess formation in any way.

My attention was particularly directed to these more or less diffuse connective-tissue formations in staphylococcus infection of the kidney by the condition found in the kidneys of a rabbit belonging to Dr. J. H. Wright, to whom I am indebted for the use of the case. The animal had received 1 cubic centimetre of a bouillon culture of the staphylococcus pyogenes aureus in the ear vein, but had shown no symptoms as a result of the injection. After it had been killed six weeks later, however, in the course of another experiment, the following renal changes were noted:

Macroscopically, the kidneys were somewhat smaller than normal, the cortex being distinctly granular and of a grayish colour. On section the entire cortex was diminished in size, the areas where the diminution was most marked corresponding to the depressions on the surface. In the pyramids there were a few oblong whitish ne-

crotic foci, the long diameters of which were parallel to the direction of the tubules. The tissue was denser and firmer than normal. No abscesses or necroses could be made out in the cortex. The kidneys presented the general appearance of the granular kidney in man. Portions were hardened in corrosive sublimate, and embedded both in celloidin and paraffin, from which sections were cut and stained with hæmatoxylin and eosin.

The pathological changes may be divided into two classes—(1) those occurring in the immediate vicinity of the abscesses, and (2) those distributed elsewhere throughout the kidney. No organisms were demonstrable in the abscesses, although the various bacterial stains were employed. The central mass of the abscess is more or less broken up, and is composed of nuclear detritus and polynuclear leucocytes. Immediately surrounding this central mass is a capsule composed of epithelioid cells with large, distinct, vesicular nuclei. Lying externally to this is a zone composed of lymphoid cells irregularly distributed through a large amount of connective tissue. Beyond these several layers, which are not sharply defined but merge more or less irregularly into each other, the interstitial tissue is filled with lymphoid cells, the tubules being widely separated from each other by a dense connective tissue rich in blood vessels and containing both epithelioid and lymphoid cells. Almost all of these tubules are partially filled with masses of hyaline material, in which pus cells are frequently embedded.

The cortex shows a very marked and diffuse increase in the connective tissue. The tubules are atrophied, and the membranæ propriæ greatly swollen. In certain areas the connective tissue seems to be almost entirely composed of the swollen membranæ propriæ of the tubules. Some of the tubules are completely collapsed, showing no lumen or epithelium, and in places there are single rows of cells surrounded by a dense membrana propria. Many of the tubules are filled with hyaline material, and the scattered epithelial cells in the tubules are not infrequently filled with hyaline droplets. The muscular coat of the blood vessels is thickened, and in some of the medium-sized arteries there is a slight increase in the tissue of the intima.

Plate XXIV shows a section through the cortex of this kidney seen under a low power. The glomeruli are small and atrophic; in places they have been completely converted into masses of dense sclerotic tissue. The atrophy of the glomeruli seems to have been induced entirely by a gradual growth of the connective tissue of the capsule. There seems to be no primary atrophic condition in the vessels of the glomerulus.

The changes produced in this kidney are of two sorts: there are focal lesions, which are undoubtedly to be attributed to the bacteria, and which are to be seen only in the immediate sphere of their action, while at the same time other lesions are apparent which are essentially diffuse, affecting all parts of the kidney, though with a greater intensity at certain places, and which, both macroscopically and microscopically, have a great resemblance to those which are observed in the granular contracted kidney in man. The abscesses differ from the acute abscesses produced by staphylococcus injections in the absence of the central masses of bacteria. It is probable that the primary necrotic foci have here gone on to complete abscess formation, the tissue having become liquefied and the greater part of the necrotic material having been removed by absorption or by passage out of the kidney through the tubules.

The focal lesions in this case are easily explained, and are certainly due to the primary presence of the bacteria. It is more difficult to explain the diffuse lesions, which consist in atrophy and degeneration of the parenchyma and increase in the connective tissue. As has been said, Ribbert observed lesions of the same character, though not of the same extent, in kidneys of rabbits which had lived for several weeks after receiving injections of the staphylococcus, and he is disposed to attribute them to a growth of the interstitial tissue following a destruction of the parenchyma. It certainly seems probable that the diffuse lesions may have been due to the prolonged action of the toxins of the injected organism, and that these toxins may have been produced in the foci of the kidney in which the bacteria first settled. It is also possible that there may have been a general distribution of small numbers of organisms throughout the kid-

ney, the lesions produced by them not taking the form of definite abscess formation.

With the view of separating these two classes of lesions, a series of experiments was undertaken in order to determine the effect produced by the toxins alone. The general plan adopted consisted in the frequent injection of small amounts of sterile, filtered bouillon cultures of the staphylococcus into the ear vein. Owing to the great difficulty encountered in obtaining virulent organisms and sufficiently strong toxic solutions, many of the experiments resulted unsatisfactorily. Three, however, gave positive results.

Experiment 1.—The staphylococcus pyogenes aureus used in this instance was obtained from an abscess of the neck. A flask of bouillon was inoculated with the second generation of this organism on April 7, 1895, and left in the thermostat until April 22d, when one half per cent of carbolic acid was added, after which the culture was filtered through porcelain. A pure culture of the staphylococcus aureus on blood serum was obtained from this bouillon on April 22d, and a rabbit, which on this date received one cubic centimetre of the bouillon in the ear vein, died in twenty-eight hours.

This perfectly clear solution of toxins was injected into the ear veins of a small but full-grown male rabbit, as follows:

| | | | |
|---|---|---|---|
| April 27 | 0·5 c. c. | June 2 | 1.0 c. c. |
| " 29 | 0·5 " | " 5 | 3·0 " |
| May 2 | 1·0 " | " 7 | 5·0 " |
| " 4 | 1·0 " | " 10 | 5·0 " |
| " 6 | 1·0 " | " 16 | 5·0 " |
| " 8 | 1·0 " | " 21 | 10·0 " |
| " 10 | 1·0 " | " 24 | 4·0 " |
| " 15 | 1·0 " | " 28 | 5·0 " |
| " 18 | 1·0 " | July 3 | 10·0 " |
| " 20 | 1·0 " | " 7 | 7·0 " |
| " 22 | 0·2 " | | |

The animal was killed July 19, 1895. During the last three weeks it had been less lively than usual, and had lost some weight. The urine was not examined. Neither the kidneys nor the other organs showed anything macroscopically abnormal.

The kidneys were hardened in corrosive sublimate, alcohol, and Flemming's solution, embedded in celloidin or paraffin, and stained with hæmatoxylin and eosin. Pieces hardened in Flemming's solution showed

no fatty degeneration. Sections made lengthwise through the whole kidney, when examined with a low power, showed a general increase in the numbers of cells in the interstitial tissue. This increase is more marked, however, over certain areas, where the tubules appear pressed apart by the abnormal number of cells.

Examination with the oil immersion showed an evident new formation of cells in the interstitial tissue between the tubules of the cortex. These cells are certainly not polymorphonuclear leucocytes. As a rule, their protoplasm can not be distinguished; but when it can be, it is of a pale colour, and varies in amount, the quantity usually being small, but occasionally considerable. The nuclei are large, round, moderately vesicular, and are certainly different from the normal nuclei of the endothelium. We were unable to determine whether or not these cells are derived from the cells of the capillaries. There is nothing, however, as will be pointed out later, in the condition of the tubules to lead us to consider this accumulation of cells as secondary to a degeneration of the epithelium.

This accumulation of cells is more marked in the vicinity of many of the glomeruli than elsewhere. The capsules often show an increased formation of cells, and sometimes there is an extension of cells along the glomerular vessels into the root of the glomerulus. The capsular spaces are, as a rule, smaller than in the normal kidney, and often contain a granular material. The vessels of the glomeruli are pervious. There is, however, an increase in the cells of the capillary loops, as we were able to show by actual count in glomeruli of the same size in sections taken from this and from the normal kidney hardened in the same way, cut of the same thickness, and stained by the same method. This increase varies from fifty to one hundred per cent. These cells are of the same character as those just described as present in the interstitial tissue of the tubules, and seem to lie in the intercapillary tissue.

The epithelium of the convoluted tubules is swollen and granular, and occasionally contains hyaline globules. The edge turned toward the lumen is usually broken and irregular, and the striated border is not apparent. Here and there the epithelial cells have been loosened from their surroundings, and broken edges of epithelium project into the tubules. These degenerative changes are most marked in the tubules leading out of the glomeruli. They are, however, no more marked in the vicinity of the cellular accumulations than elsewhere, and nowhere is there anything approaching to a total destruction of the epithelium. Some of the convoluted tubules are filled with granular masses, and in a few of the small collecting tubules of the cortex there are hyaline casts.

There is a very extensive new formation of tissue, of the same general character as that in the cortex, between the tubules of the pyramids. The tubules of Henle contain numerous granular-looking masses, which must be regarded as casts. The epithelium lining the pelvis of the kidney is unchanged. Plate XXV shows an area of cellular infiltration of the cortex from the kidney of this rabbit, as seen with a one-twelfth oil-immersion lens.

Exp. 2.—A flask of bouillon was inoculated, March 26, 1895, with the second generation of a staphylococcus pyogenes aureus obtained from an abscess of the kidney. One cubic centimetre of this bouillon was injected into the ear vein of a rabbit on March 28th. A pure culture of the staphylococcus aureus was also obtained on blood serum. One half of one per cent of carbolic acid was then added, and the solution filtered through porcelain. The rabbit emaciated gradually, and began to appear sick on April 5th. He was killed April 8th. The kidneys were large, and contained numerous abscesses, which were also present in the heart and other muscles. This solution of toxins was injected into the ear vein of a large adult female rabbit as follows:

| | | | |
|---|---|---|---|
| April 13 | 0·5 c. c. | May 15 | 1·0 c. c. |
| " 15 | 0·5 " | " 18 | 1·0 " |
| " 17 | 0·5 " | " 27 | 3·0 " |
| " 19 | 0·5 " | June 2 | 5·0 " |
| " 22 | 0·5 " | " 5 | 5·0 " |
| " 25 | 0·5 " | " 7 | 5·0 " |
| " 27 | 0·5 " | " 10 | 2·5 " |
| " 29 | 0·5 " | " 16 | 10·0 " |
| May 2 | 1·0 " | " 21 | 10·0 " |
| " 4 | 1·0 " | " 24 | 10·0 " |
| " 6 | 1·0 " | " 28 | 10·0 " |
| " 8 | 1·0 " | July 1 | 10·0 " |
| " 10 | 1·0 " | " 3 | 13·0 " |

The rabbit was killed July 12th, having shown no symptoms of disease during life. The organs showed nothing macroscopically abnormal. The urine was not examined.

The kidneys were prepared as in the previous case. The specimens show fairly numerous areas in the cortex, in which the interstitial tissue is increased. This increase is due to an accumulation of cells, less numerous, indeed, but of the same nature as those between the convoluted tubules in Case I. The tubules are compressed in some places, but show no degenerative changes. The vessels and glomeruli appear normal.

Exp. 3.—The culture of staphylococcus aureus used in this experiment was obtained from an abscess of the thumb. A flask of bouillon was inoculated with the second generation of this organism on April 7th.

A rabbit received 1 cubic centimetre of this culture in the ear vein on April 17th, and died on April 20th. The autopsy showed numerous abscesses in the kidneys and heart. One half of one per cent of carbolic acid was added to the culture, which was then passed through a porcelain filter. This toxin solution was injected into the ear veins of a large adult female rabbit, as follows:

| | | | | | |
|---|---|---|---|---|---|
| April | 23 | 0·5 c. c. | June | 2 | 5·0 c. c. |
| " | 25 | 0·5 " | " | 5 | 5·0 " |
| " | 27 | 0·5 " | " | 7 | 5·0 " |
| " | 29 | 0·5 " | " | 10 | 5·0 " |
| May | 2 | 1·0 " | " | 16 | 10·0 " |
| " | 4 | 1·0 " | " | 21 | 5·0 " |
| " | 6 | 1·0 " | " | 24 | 10·0 " |
| " | 8 | 1·0 " | July | 1 | 2·0 " |
| " | 10 | 1·0 " | " | 3 | 9·0 " |
| " | 15 | 1·0 " | " | 7 | 3·0 " |
| " | 18 | 1·0 " | " | 12 | 10·0 " |
| " | 20 | 1·0 " | " | 19 | 5·0 " |
| " | 22 | 1·0 " | " | 22 | 5·0 " |
| " | 27 | 2·0 " | | | |

The rabbit emaciated rapidly during the last few weeks, during which she received the injections, and about August 1st became very sick. She was killed August 5th, as she was then moribund. The kidneys showed nothing macroscopically abnormal. They were prepared as in the two previous cases. Microscopically, they showed similar but less marked and less extensive changes than those demonstrable in the kidneys of the second experiment.

The lesions of the kidneys produced by bacteria have been carefully studied by many investigators during the last ten years. Ribbert * has devoted especial attention to those produced by the staphylococcus aureus. Pernice and Scagliosi † injected guinea-pigs with filtrates of the anthrax bacillus and of the bacillus pyocyaneus. The animals were killed in forty-eight hours. In general, the results were almost negative. In some cases, however, hæmorrhages were found in the interstitial tissue. The epithelium was as a rule unchanged. Here and there, however, it showed granular degeneration. The epithelium of some of the straight tubules was desquamated and in

* Ribbert, Die pathologische Anatomie und die Heilung der durch den Staphylokokkus Aureus hervorgerufenen Erkrankungen, Bonn, 1891.

† Virchow's *Archiv*, Bd. cxxxviii, p. 521.

some cases the walls were collapsed. With this exception, I am unable to find that any attempt has been made to study the renal lesions produced by bacterial toxins alone. Certainly no one has made a careful study of the effects of continued and repeated injections of such toxins.

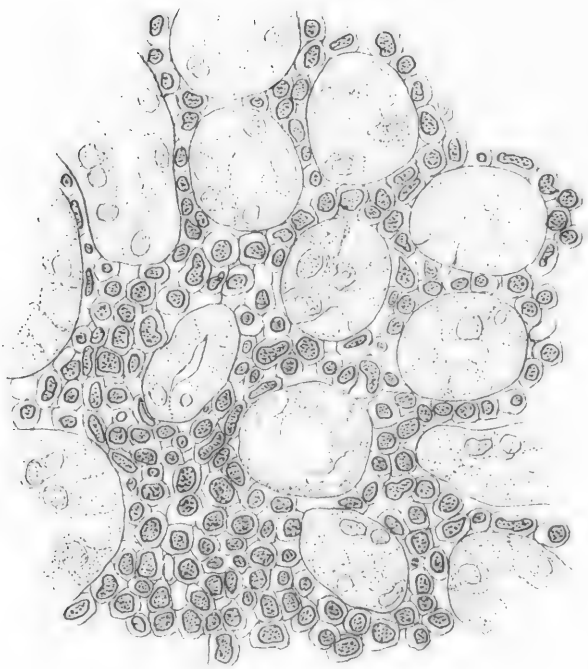
The positive results obtained in these three animals seem sufficient to prove that a chronic interstitial process may be caused in the kidneys by the action of the toxic products of bacteria. It must be admitted that the human kidney is more or less constantly exposed to the action of chemical substances produced by bacteria. Hence it would seem justifiable to assume that a certain proportion of the cases of chronic interstitial nephritis in man may be due to the action of such substances.

These investigations were carried on in the Sears Pathological Laboratory of the Harvard Medical School, under the direction of Prof. W. T. Councilman, to whom I am indebted for much valuable advice and assistance.

## Description of Plates.

Plate XXIV.—Section of cortex of kidney of rabbit killed six weeks after injection of culture of staphylococcus pyogenes aureus into the ear-vein. Low power. Description in text.

Plate XXV.—Section of cortex of rabbit's kidney of Experiment 1, showing an area of cellular infiltration. Oil immersion, $\frac{1}{12}$. Fuller description in the text.



# INFLUENCE OF ACUTE ALCOHOL POISONING ON NERVE CELLS.

By COLIN C. STEWART,
FELLOW IN PHYSIOLOGY, CLARK UNIVERSITY.

(*From the Physiological Laboratory of Clark University, Worcester.*)

PLATE XXVI.

In 1895 Dehio * demonstrated changes in the ganglion cells of the cerebellum produced by acute alcohol poisoning. In his research eight rabbits, of which two were controls and one, being diseased, was useless, formed the first series for experiment. Alcohol was administered by the stomach and by subcutaneous injection, the latter method producing the greatest result in the shortest time. The usual dose was 7 to 10 cubic centimetres of ninety-six per cent alcohol, reduced with water to about forty per cent, followed, as consciousness returned, by a further dose of 5 cubic centimetres, until in all 20 or 25 cubic centimetres had been given. Death occurred in from one to thirty-four hours, according to the amount given, though the rabbit living thirty-four hours was allowed to recover during the night.

Slides were prepared by Nissl's methylene-blue method. The alcohol effect was not observed with any certainty in those animals which died in the earlier stages of intoxication, while the others showed the following definite changes in the appearance of Purkinje's cells: The cells showed, instead of the normal, fine-meshed, stained network, fine irregularly arranged granules of approximately uniform size, while the normally unstained substance had taken on a pale blue tone. This change affected sometimes the whole of the cell, sometimes a part only, while nucleus, nucleolus, and the granules of

* Heinrich Dehio, Experimentelle Untersuchungen über die Veränderungen der Ganglienzellen bei der acuten Alkoholvergiftung. *Centralbl. für Nervenheilkunde und Psychiatrie*, 1895, N. F., vi, 113.

the cell processes remained unchanged. By no means all the ganglion cells were affected. In most cases whole rows of normal cells were observed, with pathologically altered cells scattered singly or in groups. Other parts of the central nervous system were studied in the same way, but nothing was observed to warrant any conclusions as to experimentally induced changes.

A second series included three dogs, one living five hours, one thirty hours, and one a normal control. The one living five hours showed nothing, but the one intoxicated for thirty hours gave more pronounced results than the rabbits.

With the hope of confirming and, if possible, extending Dehio's results, three adult male cats were chosen, and absolute alcohol was given to two of them, according to the following scheme:

| | Cat I. | Cat II. | Cat III. |
|---|---|---|---|
| May 27th. | ..... | ..... ..... | 2.45 P. M.: 12 c. c. absolute alcohol, diluted to 10 per cent with a 0·6 per cent salt solution, injected into the abdominal cavity. |
| | | | 3 P. M.: 12 c. c. more, in the same way. |
| May 28th. | ..... | ..... | Under the influence of alcohol all day; partially recovered during the following night. |
| May 29th. | 7.30 P. M.: Killed by decapitation. | 6 P. M.: 22·5 c. c. of absolute alcohol, 50 per cent solution in 0·6 per cent salt solution, injected into the abdominal cavity. Stupor commenced before the alcohol was all injected. | 8.45 A. M.: 8 c. c., given as before. |
| | | | 2 P. M.: 8 c. c., given as before, neither dose quite sufficing to produce complete unconsciousness. |
| | | | 8.45 P. M.: 20 c. c. absolute alcohol, 50 per cent solution in 0·6 per cent salt solution, injected as before. |
| | | | 9 P. M.: 5 c. c. of the same injected subcutaneously, repeated after 5 minutes. |
| | | 6.50 P. M.: Cat dead. | 9.15 P. M.: Cat dead. |

Blocks of tissue of equal size from corresponding portions of the right cerebral hemisphere and vermis, and from the spinal cord opposite the sixth pair of cervical spinal roots, were excised and placed immediately in one common vessel of absolute alcohol. Next morning, about twelve hours later, this was replaced by fresh alcohol, and on the following day, after about thirty-six hours, sections of a uniform thickness of 10 micra were cut under absolute alcohol and placed in separate dishes of fresh alcohol.

In staining, every endeavour was made to have specimens to be compared subjected to exactly the same conditions. Three corresponding sections, one from each animal, were lifted to a glass slide, drained, stained in the same large drop of methylene-blue solution, drained again and placed together in the same dish of anilin alcohol, washed, dehydrated and cleared together, and mounted under the same cover slip. So that with the single exception of placing the cut specimens in exactly similar dishes in the same alcohol between the time of cutting and the time of staining, *any three comparable sections were treated as one specimen.*

The sections were stained for from forty seconds to five minutes in the following solution:

| | |
|---|---|
| Methylene-blue, Grübler, B. Pat. | 3.75 |
| Sapo. venet. | 1.75 |
| Distilled water. | 1,000. |

Slightly better results were obtained if the slide carrying the pool of stain in which the sections lay was warmed until steam was given off. After staining, the sections were bleached in

| | |
|---|---|
| Anilin oil | 10 |
| Absolute alcohol | 90 |

for five or ten minutes, according to the depth of the stain. The anilin oil was then washed out in two or three washes of absolute alcohol, after which the sections were cleared in xylol and mounted in xylol balsam.

A variation in the intensity of the stain of any three sections of a comparable set was apparent from the first to the naked eye. The normal stained always bluer than the second of the series, and the second always bluer than the third or most heavily poisoned. A microscopical examination of the mounted specimens showed that while this variation in depth of tone was due in the cerebrum to variations in the tone of the cortical cells themselves, and of the Purkinje cells and cells of the granular layer in the cerebellum, yet in the gray matter of the cord it was produced in great measure by changes in the nuclei of the smaller cells. The large multipolar cells in the cord in both the pathological specimens, however, gave evidence of a great

decrease in the number of their chromophile granules (see Plate XXVI, Figs. 10, 11, 12), and the cells from the cord of the animal under the influence of alcohol for the longest period of time (fifty-four hours and a half) show fewer granules than those from the animal dying in fifty minutes.

There are, of course, as was true of Dehio's * specimens, some cells unaffected and some not so much so as others. To give an approximate expression of the proportion of pathological cells a scale was chosen corresponding to the scale of the three multipolar cells drawn for the accompanying plate, and the cells of the slide from which the drawings were made were counted and classified according to that scale. The following table will show the result of that classification:

| | Class 1. (See Fig. 10.) | Class 2. (See Fig. 11.) | Class 3. (See Fig. 12.) |
|---|---|---|---|
| | Per cent. | Per cent. | Per cent. |
| Cat I, normal, 136 cells | 30·9 | 41·9 | 25·2 |
| Cat II, 50 minutes, 107 cells | 24·3 | 31·8 | 43·9 |
| Cat III, 54½ hours, 115 cells | 5·2 | 29·6 | 65·2 |

In the cerebellum (see Figs. 7, 8, and 9 of Plate XXVI) a great decrease in the number of granules in the pathological cells is also to be observed. All the Purkinje cells from the three specimens on the slide from which the drawings have been made were counted and classified according to the scale which the drawings show. The following is a tabulation of the result:

| | Class 1. (See Fig. 7.) | Class 2. (See Fig 8.) | Class 3. (See Fig. 9.) |
|---|---|---|---|
| | Per cent. | Per cent. | Per cent. |
| Cat I, normal, 356 cells | 81·2 | 18·8 | .... |
| Cat II, 50 minutes, 286 cells | 1·1 | 72·4 | 26·5 |
| Cat III, 54½ hours, 309 cells | 0·7 | 54·7 | 44·6 |

The cerebellum gives much more definite results than the cord, for, whereas in the section from the normal cat we have only 18.8

* Dehio, *loc. cit.*

per cent of the cells showing even a moderate deficiency in granules, we have in the two pathological sections only 1.1 per cent and 0.7 per cent respectively which can be compared with the normal cells as to granulation; and, too, the section from the animal receiving alcohol for the longer time shows in its cells a greater decrease in granules than the other.

To estimate, if possible, the extent of other changes in the ganglion cells of the cerebellum, measurements were made for twenty nucleated cells in each section, those cells being chosen which were nearest to the cell drawn for the plate from each section. These measurements will be given with the reservation that they may be an expression of variations due to original differences in the animals used in the experiment.

| | Average long diameter of cell. | Average transverse diameter of cell. | Average mean diameter of nucleus. | Average diameter of nucleolus. |
|---|---|---|---|---|
| Cat I, normal | 30·1μ | 20·95μ | ..... | 2·94μ |
| Cat II, 50 minutes | 28·3μ | 23·45μ | 13·15μ | 2·85μ |
| Cat III, 54½ hours | 29·1μ | 17·4μ | 11·25μ | 2·76μ |

It was found impossible to measure the nuclei of the cells in the section from the normal cat with an oil-immersion lens, on account of the heavy granulation always present. Very little importance, too, can be attached to the measurements of the cell diameter, for a small difference in the plane of the section would make an enormous difference, more especially in the apparent transverse diameter of the cell. But measurements of the nucleoli are absolute, and were taken accurately, so that the serial decrease in diameter must be due either to a constant original difference in the research animals or to an alcohol effect, equally constant, which must be decided by further experiment.

In the cerebrum almost all of the cells of a section are of the type shown in the corresponding figures of Plate XXVI. Figs. 1 and 2 are from the normal cat; Figs. 3 and 4 are from Cat II; Figs. 5 and 6 are from Cat III. So few exceptions were found that a count was considered unnecessary. No attempt was made to measure nuclei or

nucleoli, on account of the difficulty of selecting cells that could be accurately compared; but if further experiment should show the importance of such measurements, the specimens will be gone over again for that purpose.

No alteration of the nucleus or disintegration of the nucleolus, such as Berkley * describes for chronic alcoholism in rabbits, has been observed. Fig. 11 shows what appeared to be vacuolation of the nucleolus—the only such case found.

Preparations by the quick Golgi method, and by the phosphomolybdic-acid method used by Berkley, showed nothing conclusive as to the presence of moniliform swellings of the cell dendrites and degeneration of contact granules, such as are described by Andriezen † and Berkley ‡ for chronic alcoholism.

With regard to the clearness of results obtained in this experiment, it may be noted that Dr. Hodge,# in his work on fatigue of nerve cells, obtained always much clearer demonstrations of the fatigue effect in cats than in rabbits or frogs.

Since the results obtained in this experiment are a confirmation and extension of Dehio's work, already referred to fully, it has been thought better to publish. With regard to the significance of the changes described, much more can possibly be said when the methylene-blue staining method has been applied for the demonstration of normal and abnormal fatigue effects in the nerve cell in a series of experiments already contemplated for the laboratory of Clark University.

This experiment is one of a series on the physiology of alcohol now being carried on for the Committee of Fifty in the Physiological Laboratory of Clark University, under the direction of Dr. C. F.

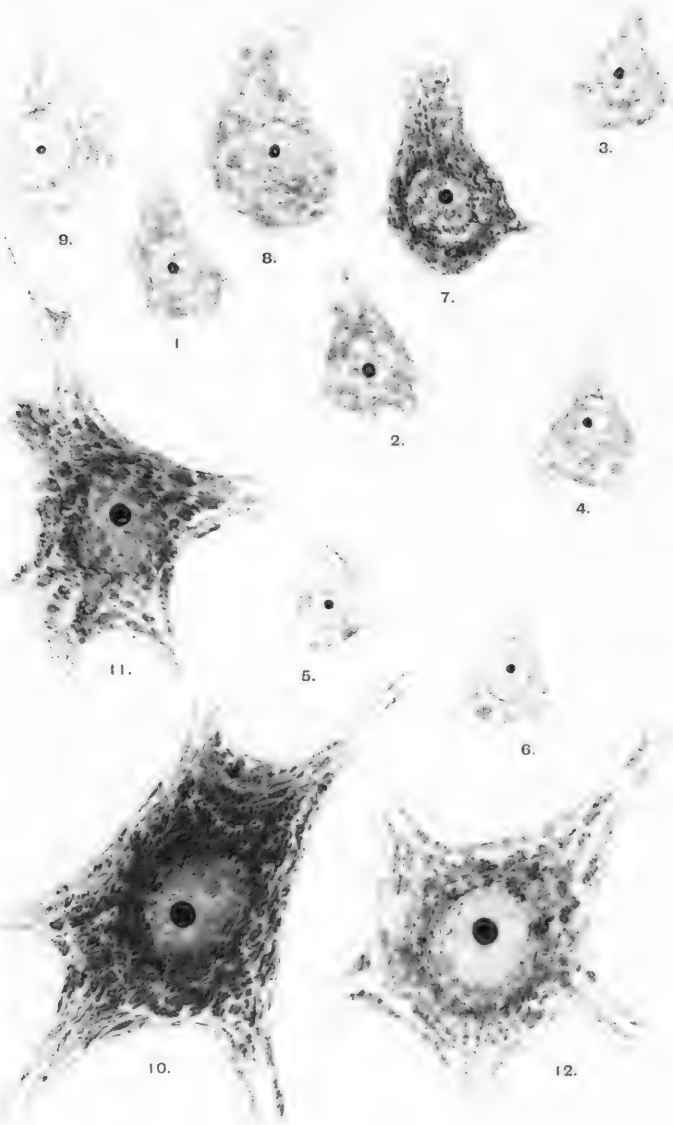
* Henry J. Berkley, Studies of the Lesions Produced by the Action of Certain Poisons on the Cortical Nerve Cell. I. Alcohol. *Brain*, Part LXXII, Winter Number, 1895, p. 473.

† W. Lloyd Andriezen, On Some of the Newer Aspects of the Pathology of Insanity. *Brain*, Part LXVIII, Winter Number, 1894, p. 668.

‡ Henry J. Berkley, *loc. cit.*

# C. F. Hodge, Ph. D., A Microscopical Study of Changes Due to Functional Activity in Nerve Cells. *Journal of Morphology*, vol. vii, No. 2.

NOTE.—Dr. Hodge kindly permits me to say that rabbits were used in his experiments, though results were not reported.—C. C. S.




A. Hoen & Co.

Hodge, for whose kind help and many valuable suggestions I must express my sincere gratitude. My thanks are especially due, also, to Mr. Jonas G. Clark, of Worcester, without whose generous provision for scientific investigation the work would have been impossible.

## Explanation of Plate XXVI.

Figs. 1, 2, 3, 4, 5, and 6.—Large pyramidal cells from the central cortex. Figs. 1 and 2 are from Cat I, normal; 3 and 4 are from Cat II, alcohol for fifty minutes; 5 and 6 are from Cat III, alcohol for fifty-four hours and a half.

Figs. 7, 8, and 9.—Purkinje cells. Fig. 7 is from Cat I, normal; 8 is from Cat II, alcohol for fifty minutes; 9 is from Cat III, alcohol for fifty-four hours and a half.

Figs. 10, 11, and 12.—Multipolar cells from the spinal cord. Fig. 10 is from Cat I, normal; 11 is from Cat II, alcohol for fifty minutes; 12 is from Cat III, alcohol for fifty-four hours and a half.

Drawings are made with Zeiss Oc. 6, and homog. imm. lens, 2 millimetres, aperture 1.30. Outlines were drawn with Zeiss camera lucida after Abbe.

# OF THE ACTION OF ETHER ON CONTRACTURE AND OF POSITIVE KATHODIC POLARIZATION OF VERTEBRATE VOLUNTARY MUSCLE.

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INSTRUCTOR IN PHYSIOLOGY IN THE HARVARD MEDICAL SCHOOL, BOSTON, MASS.

PLATES XXVII–XXX.

By reasons which will be discussed in a later part of this paper and because it seemed that the action of such a substance would be of interest in connection with the study of contracture, I have been led to investigate the effect of ether on the form of contraction in response to a single stimulus of muscles which have been exposed to the influence of veratrine, of 0.6-per-cent sodium-chloride solution, and of dehydration. The effect of all these is to change the simple twitch of a normal muscle into a contraction enormously increased in amount and duration. Is the altered condition of the muscle, of which this abnormal form of response to a single stimulus is the outward and visible sign, stable as long as contractility persists under etherization, or is there associated with the gradual loss of contractility a change in the form of its expression—a return to the normal twitch of short duration?

## I. The Action of Ether on Veratrinized Muscles.

### GENERAL CONSIDERATIONS REGARDING METHOD.

Since, as has long been known, stimulation frequently repeated has itself an effect on the form of contraction of veratrinized muscle, it is evident that the intervals between successive stimuli must be long enough to exclude this source of error. On the other hand, in order to obtain a correct idea of the action of ether upon the form of

contraction, it is highly necessary to apply as many stimulations to the muscle as possible before its contractility is extinguished by this substance. Preliminary experiments showed that intervals of not less than one minute are required to prevent too rapid falling away of the contracture. It is evident that, on account of this falling away, observations made during the muscle's recovery from etherization, regular stimulation being kept up through the whole experiment, are more convincing of an action of ether on the form of contraction than those made while the muscle is being etherized.

In the first experiments made the curarized and veratrinized sartorius muscle was etherized in various relatively small moist chambers, while induction shocks were passed at regular intervals through it from end to end through the bones with which it is in connection. Under these circumstances it was found that loss of contractility took place too speedily for the changes in the form of contraction of the muscle to be made out, if the necessary interval between successive stimulations was observed. Remembering, however, that a sartorius immersed in 0.6-per-cent sodium-chloride solution is acted on more readily at its tibial than at its pelvic end,* it occurred to me that under the action of ether both ends might become sooner unirritable than the rest of the muscle, and cause a "*polares Versagen*"—a failure of induction shocks sent through the muscle to stimulate it, owing to the local inexcitability of physiological kathode, perhaps also of anode. To avoid this, two electrodes of fine copper wire were made to lightly clip the muscle at the ends of its middle third. When the muscle was stimulated through these it was found to respond long enough to stimulation when etherized for the action of the vapour on the form of contraction to be made out.

I convinced myself directly by special experiments on the unveratrinized curarized sartorius, subjected to the action of ether vapour and stimulated by induction shocks sent in both directions through its whole length and through its middle third, that the ends lose their excitability first, and that we have results corresponding exactly with

*F. S. Locke, Die Wirkung der physiologischen Kochsalzlösung auf quergestreifte Muskeln. *Arch. f. d. ges. Physiol.*, liv, 501, 1893.

those obtained with a 0.1-per-cent solution of potassium chloride on the muscle in question,* the tibial end being the more readily acted upon by the ether than the pelvic end. As etherization proceeds, the normally higher contractions called forth by induction shocks descending from pelvic to tibial end in the muscle become lower than those called forth by ascending shocks, the effect of the greater current density at the slender tibial end being annulled. As etherization proceeds, the contractions called forth by descending shocks continue to decrease in height more rapidly than those called forth by ascending shocks, and are the first to cease to appear. Ascending shocks from end to end become next ineffective, the continuity of the muscle remaining irritable for from two to three minutes after this occurs. It may be added that if the muscle is allowed to recover from the action of the ether, ascending shocks become effective sooner than the descending shocks, the latter, however, after recovery is complete, becoming once again the more effective.

In these experiments on curarized muscles I uniformly found the muscle much more excitable to shocks sent through its middle third than to shocks, even when descending, passed through its whole length. This result is readily explained by the lower electric excitability of the nerveless ends of the sartorius even when curarized—a fact established by Kuehne † and Pollitzer ‡—and by the greater electric resistance of the whole muscle, with its tendons and stumps of bone left attached to them.

This difference of resistance was of course not present in the experiments of Kuehne and Pollitzer, in which the excitability of the muscle at different points of its length was investigated with induction shocks

* F. S. Locke, *op. cit.*, p. 510. The action of 0.1-per-cent potassium-chloride solution there described was due not merely to the potassium chloride, but also to the hypotonicity of the solution, causing it to act to a very high degree like pure water. This, fortunately, does not vitiate the demonstration by the help of this solution that the thinner tibial end of the sartorius is more susceptible than the pelvic end to the influence of media surrounding the muscle.

† Kuehne, *Arch. f. Anat., Physiol. u. wissensch. Med.*, 1860, 477.

‡ Pollitzer, *Journ. of Physiol.*, vii, 274, 1886.

passed obliquely through it by means of transverse wire electrodes placed a short distance apart, one on each side of the muscle. It may be pointed out here, however, that the conclusion drawn by Pollitzer from his results, that curare leaves unaffected certain nerve elements in the muscle, can not be well founded. He supposes that the excitation of these nerve elements ("the parts peripheral to the last node of Ranvier") explains the greater excitability of the parts of the muscle containing such. But, if this were the case, these nerve elements would be also excitable in a curarized muscle by currents passed through its whole length, and it would be as impossible for injury of the ends of such a muscle to render it inexcitable to currents entering and leaving it through injured tissue ("*polares Versagen*," Biedermann, Engelmann, and van Loon), as it actually is in the case of uncurarized muscles (van Loon). The failure of electric excitation caused by merely polar injury of a curarized muscle is to me the absolute proof that curare does not leave in physiological connection with the contractile material any excitable structures that do not traverse the muscle uninterruptedly from end to end. It is therefore, I think, the strongest possible proof of the independent irritability of muscle, as an argument for which I do not know it to have yet been used, and the results obtained by the electrical stimulation of curarized muscles described in this paper may be safely regarded as due to the action of the stimulus on muscle and on muscle alone.

My ultimate mode of experiment was as follows: The sartorius of a completely curarized grass-frog, into the dorsal lymph sac of which a few drops of a one-per-cent solution of veratrine acetate had been injected from six to fifteen minutes before the preparation of the muscle, was fixed in the muscle-chamber,* and the electrodes of fine copper wire were placed upon it. The muscle was first minimally stimulated, and was always found to give minimal contractions

* The chamber used is illustrated in the Cambridge Scientific Instrument Co.'s catalogue (p. 75, 1891), and measures approximately $12 \times 9 \times 6.5$ centimetres. It had been somewhat modified, and an ivory pulley 6 millimetres in diameter was fixed to the axis of the muscle lever, around which the thread carrying the load was slung. The distance between the axis and point of pull of the muscle was 40.5 millimetres; the length of the whole lever, 148 millimetres. The weight of the whole lever, with all its attachments except the actual load, was 1.05 gramme. When horizontal, it exercised a traction downward of 0.8 gramme along the thread connecting it with the muscle.

of long duration (Plate XXVII, Figs. 1A and 3A). The secondary coil of the inductorium was then moved up toward the primary, and regular stimulation with opening shocks commenced. The length of the interval between successive shocks was determined by the time of rotation of the drum, which in different experiments varied from one to three minutes. The successive curves of contraction were either superposed, in which case closing and opening of the primary circuit was effected by the passage through a mercury meniscus of an amalgamated copper wire rotating with the drum (the closing shocks were cut out by an *Abblender*), or followed one another in *imbrication latérale*, in which case closing and opening were effected by hand. After three or four typical veratrine curves had been recorded, a small piece of folded lint, on which about a cubic centimetre of ether had been poured, was placed in the moist chamber, and allowed to remain there until the muscle's irritability was sufficiently diminished. After its removal, the cover of the moist chamber before being replaced was well washed out with tap-water to get rid of ether clinging to it, the periodical stimulation being kept up as long as appeared necessary.*

THE ACTION OF ETHER ON THE FORM OF CONTRACTION OF VERATRINIZED MUSCLE.

There is no necessity to describe here the well-known general features of the action of veratrine on voluntary muscle. I will only draw special attention to the character of the contraction curves of the fresh veratrinized sartorius, which show that, contrary to the

* It is not always unavoidably necessary to send the stimulating shocks only through the middle third of the muscle in order to show clearly the effect of etherization on the veratrinized sartorius. This is occasionally possible, as I have found since the above was written, with the shocks sent from end to end of the muscle in the ordinary way. This result is accounted for by the fact that, as was found in the experiments made in the manner described in the text, muscles are occasionally met with which are very refractory to ether, and remain well irritable in an atmosphere saturated with it for as long as fifteen minutes. To avoid loss of time and material it is, however, advisable to take advantage of the difference in behaviour of the ends and of the continuity of the muscle above described.

opinion usually held, the action of the poison is by no means principally exerted during the period of relaxation of the muscle, the period of shortening being but little affected, but that the latter is also markedly modified, the amount of shortening being enormously increased, so that this phase of the contraction resembles that of a maximal tetanus rather than that of a normal twitch in answer to a single stimulation. It must be added, also, that the contraction curves of the fresh veratrinized sartorius stimulated at sufficiently long intervals show none or but slight traces of the characteristic and well-known dicrotism.

The action of ether on the form of contraction of veratrinized muscle is shown so clearly in the curves accompanying this paper that the verbal description of it will be made as short as possible.

The progressive diminution in the height of the contraction, which takes place under etherization, is accompanied also by an alteration in its form. A beginning of this is usually visible in the first contraction of the muscle after etherization has been begun, its summit being sharper and relaxation more rapid. The next contraction may be an almost normal twitch, followed only by a slight contraction-remainder. If, however, etherization be not so rapid, or the muscle be more profoundly veratrinized, the changes in the form of contraction may not be so abrupt, and the transitional forms from spasm to twitch are seen to be of the dicrotic type, the further action of the ether being especially exerted on their second summit, converting it into a mere contraction-remainder, which at last becomes minimal and succeeds a normal twitch.

The more profoundly the muscle has been veratrinized the more must the action of the ether be pushed before the effect of the veratrine is annulled, the simple twitches then obtained not being of great height. They are always, however, absolutely different in character from the prolonged minimal contractions evolved by minimal stimulation at the commencement or during the course of the experiment before etherization has been begun.

If etherization be put an end to when the stage of the simple twitch has been reached, the contraction curves of the muscle during

its gradual recovery of contractility are generally more instructive than those recorded during etherization. In successive contractions the contraction-remainder becomes greater and greater, while the duration of maximal contraction is usually more and more prolonged. The contraction-remainder may change into a secondary hump, which rises higher and higher till it overtops the initial shortening from which it is separated by the characteristic notch. In other cases, especially those of slightly veratrinized muscles, the notch may not be so manifest.

The effect of veratrine on the form of contraction never again becomes so marked as it was before etherization of the muscle. This is principally due to the effect of the repeated stimulation, and not to an after-effect of the ether—a fact which was proved by control experiments in which veratrinized muscles without being etherized were periodically stimulated in a similar manner. The same effect of repeated stimulation comes out in the etherization experiments if sufficiently prolonged, the veratrine spasms continuously decreasing after the maximum of recovery from ether has been reached. The etherization, if it has not been pushed too far, has but little permanent bad effect on a muscle, which, if allowed to remain after a first experiment for from thirty to forty-five minutes in Ringer's solution, shows once more the action of veratrine markedly, and can be used for a second etherization.

If the etherization has been too profound, the muscle may recover only imperfectly from it, to the extent of giving poor simple twitches, the veratrine effect not reappearing.

## II. The Action of Ether on Muscles under the Influence of Physiological Salt Solution.

I have already described * the alterations of the normal form of contractions of voluntary muscle brought about by the treatment of it with 0.6-per-cent sodium-chloride solution. These are essentially similar to those caused by veratrine, the differences being of degree

* Locke, *Arch. f. d. ges. Physiol.*, liv, 501, 1893.

and not of kind. The relaxation from the state of spasm following a single stimulation is, in the case of the muscle treated with salt solution, usually more rapid than that of the veratrinized muscle. The only addition I will now make to my previous account is that, using chemically pure salt-solution in fairly large amount (100 cubic centimetres) in which to immerse the curarized or uncurarized sartorius muscle, I find that the condition of the frog has by no means the importance which I found it to possess in my earlier work. With good or poor muscles the spasm-conditioning action of the solution is well marked at all seasons of the year.

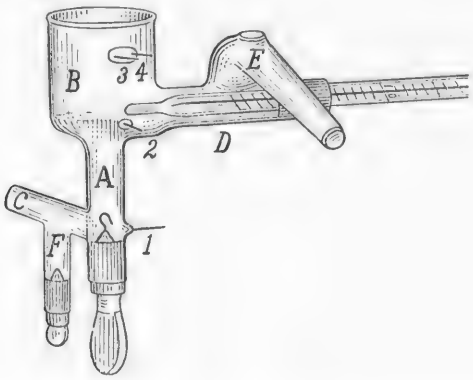
METHOD.

For the purpose of examining the action of ether on the form of contraction of muscles under the influence of physiological salt solution I have not etherized the muscle in a moist chamber, after an immersion in salt solution for from ten to thirty minutes, but have made use of a method which I have now long employed for the investigation of the action of various solutions on voluntary muscle, and which, on account of its convenience and general utility, will here be briefly described.

The objects of the method are to obtain a graphic record, as perfect as possible, of the contractions, weak and strong, of a sartorius muscle exposed to the action of a large quantity of fluid, the nature and temperature of which can be readily varied, and to enable one, with the help of only ordinary appliances, to strongly stimulate electrically the muscle without removing it from the fluid surrounding it. The use of a muscle-chamber of peculiar construction renders these objects easily attainable.

The muscle-chamber, shown in the figure (p. 638) two thirds its real size, is blown from glass tubing, and may be described as consisting essentially of a tube of varying calibre, the lower part of which (A) is of small (7.5 millimetres) diameter, to enable sufficient density of electric current to be readily obtained in the muscle, surrounded as it is by the fluid contained in the tube. The upper part (B) is of much larger (22 millimetres) diameter, in order to allow a certain amount of lateral play to the thread

connecting the muscle with the recording lever above it, so that there will be no risk that the thread will be drawn to and fixed, as far as the transmission of the movements of a lightly loaded muscle is concerned, in the edge of the fluid's meniscus. The muscle-chamber is closed below with a glass stopper, not ground in, but made to fit securely by means of a bit of rubber tubing of suitable size forming a packing between stopper and tube. A platinum hook attached to the stopper fixes the muscle in the way described below. Just above the stopper a side tube (C) acts as inlet for the slow stream of fluid maintained through the muscle-chamber. This flows out through the side tube attached to the upper part of the chamber (D), past the bulb of a thermometer fixed in this tube by a collar of rubber tubing, and then away through the bent tube (E), a hole blown at the top




of the bend of which prevents siphoning off. The bulb of the thermometer, which projects a little into the chamber, thus always remains under the surface of the fluid surrounding the muscle. The platinum electrodes 1 and 2 are used for direct stimulation; the platinum electrodes 3 and 4 for indirect stimulation. The latter are just above the surface of the fluid in the chamber. The tube F, usually closed by a rubber and glass stopper, serves to empty the chamber of fluid when required.

The sartorius is prepared for experiment in the following way: A fine silk ligature having been tied to its tibial tendon, and the muscle having been dissected out in connection with its half of the pelvic symphysis, the margin of the latter, except where the muscle is attached, is cut away so that but little more than the acetabulum is

left, and through the cartilaginous centre of this a hole is bored with a needle or pin. Through this hole the platinum hook fixed in the stopper closing the lower end of the muscle-chamber is passed, and, suspended by this, the muscle is allowed to hang down. A small pair of bulldog forceps is attached to the silk thread, its weight rendering easy the introduction of the muscle into its inverted chamber.

The chamber is now fixed in a small clamp underneath the recording lever, which consists of half a split straw attached to a Cambridge Scientific Instrument Company's "adjustable lever support," to the axis of which a small ivory pulley has been fixed, round which is slung a weight so as to counterpoise the lever. This method of counterpoising the lever has for advantages over the use of an elastic thread ease and permanency of adjustment.

The inlet tube of the chamber is connected by rubber tubing and branched glass tubing with three aspirating bottles, one of which contains Ringer's solution, another pure salt-solution (0.6 per cent), and the third pure salt-solution to which one or two per cent of ether has been added. The muscle is first stimulated when surrounded by Ringer's solution. Then a slow stream of pure salt-solution is established, and after the muscle has been exposed to its action a sufficient length of time for the alteration in the form of contraction to be marked, stimulation with strong opening induction shocks at constant intervals is commenced and maintained, a stream of ether-containing salt-solution being substituted for the other whenever it is thought necessary. The time at which the solution is turned on or off does not, of course, coincide with the change of solution in the muscle-chamber, which takes place a little later at a time not accurately determined and dependent on the rate of flow.

### THE ACTION OF ETHER ON MUSCLES UNDER THE INFLUENCE OF PHYSIOLOGICAL SALT SOLUTION.

If, in the apparatus described, the sartorius muscle, curarized or uncurarized, and as lightly loaded as possible, be surrounded with Ringer's solution or with 0.6 per-cent salt-solution made with New River tap-water, in the vast majority of cases, especially if the tem-

perature of this solution be not too high—i. e., above 20° C.—it remains absolutely quiet, and when stimulated gives a simple twitch of short duration, not leaving behind it a contraction-remainder. But if a slow stream of pure 0.6-per-cent salt-solution be passsed through the muscle-chamber, after a short but variable time the muscle begins to contract without further stimulation in the manner which Ringer * has already described. If five to fifteen minutes after the stream of pure salt-solution has been begun the muscle be electrically stimulated as before, it responds not with a twitch but with a spasm, more or less prolonged, and more or less irregular in character (Plate XXVIII, Figs. 4 and 5B). In many cases the "spontaneous" contractions appear to be superposed on the response to electrical stimulation. This need not be described further here.

More or less similar spasms appear in answer to further stimulations at constant intervals, while between them the spontaneous contractions break the abscissa. If now salt-solution containing ether (one to two per cent) be substituted for the pure normal salt-solution before employed, the first effect is seen to be a dying away of the "spontaneous" contractions, and loss of the tonic shortening of the muscle associated with them. The writing point now draws an unbroken line, and the next electrical stimulus calls forth usually only a simple twitch, the relaxation after which is complete.

Further action of the ether-containing salt-solution produces a lessening of the contractions due to electrical stimulation. If now the etherization be discontinued and a return be made to pure salt-solution, after some time both spontaneous contractions and spasms in answer to electrical stimulation return, but usually not to so great a degree as before etherization.

The spasm on electrical stimulation of the muscle due to salt-solution yields much more easily to etherization than that due to veratrine. The contractility of the muscle is very little affected at the time when it no longer gives spasmodic contractions. Its contractility may, in fact, be still increased when compared with that exhibited by the muscle when surrounded by Ringer's solution.

* Ringer, *Journ. of Physiol.*, vii, 291, 1886.

The above general description holds good for the majority of experiments conducted in the way described. In certain experiments, however, made in very cold weather, I found that 0.6-per-cent sodium-chloride solution acting on curarized sartorius muscles brought about extremely well-marked contractures, but had hardly any chemically stimulating action, whereas both kinds of effects were as usual produced on non-curarized muscles. This fact indicated that the "spontaneous" twitching of the muscles in the solution was brought about, at any rate at low temperatures, more especially by the action of the solution on the nerve endings, while its action on muscle itself was responsible for contracture. This supposition was supported by the few experiments I was able to make at higher temperatures with curarized muscles acted on by 0.6-per-cent sodium-chloride solution, containing 0.02-per-cent curare, to prevent the washing out of the poison from the nerve endings (Fig. 5). As, however, Ringer * concluded from his experiments with curare that the chemically stimulating action of salt solution is exerted on muscle itself, I do not care at this time to draw definite conclusions from my available material.

I will add here, too, that I have made etherization experiments with sartorius muscles acted on by 0.6-per-cent sodium-chloride solution, containing 0.02 to 0.06-per-cent $BaCl_2{}^2H_2O$. I did not find, however, that the addition of the barium salt to the sodium-chloride solution increased the contracture-conditioning action of the latter on the sartorius. In some cases, indeed, the opposite effect seemed to be produced. Etherization produced in these experiments also its typical effect (Plate XXVIII, Fig. 6).

### III. The Action of Ether on Dehydrated Muscles.

The remarkable effect on frog's voluntary muscle of local pressing, of drying by exposure to air, and of abstraction of water by means of glycerin injected into the animal's dorsal lymph sac have been described by Kuehne,† Biedermann,‡ and Langendorff # respectively. That diminution of the fluid content of the muscular tissue

* Ringer, *op. cit.*, p. 305.

† Kuehne, *Zeitschr. f. Biol.*, xxiv, 383, 1888, xxvi, 203, 1890.

‡ Biedermann, *Wiener Sitzungsb.*, xcvii (3), 145, 1888. See also Grünhagen, Funke's Lehrbuch der Physiologie, 6 Aufl., i, 679, Leipzig, 1876.

# Langendorff, *Arch. f.* (*Anat. u.*) *Physiol.*, 1891, 480.

is the essential common factor in the three cases is in the highest degree probable.* Of the two most striking features presented by such muscles, true secondary excitation of muscle by muscle, and response to simple stimulations by violent spasms of long duration, essentially analogous to those conditioned by veratrine and salt solution, the latter is of importance here. I have thought it worth while to investigate the effect of etherization on the form of contraction of sartorius muscles of curarized frogs into whose dorsal lymph sacs 1 to 2 cubic centimetres of glycerin had been injected an hour to an hour and a half before the preparation of the muscles.

The experiments were conducted in exactly the way before described in the case of veratrinized muscles.

Langendorff has already published graphic records of the prolonged spasmodic contractions of the muscles of glycerined frogs in response to single stimulations. I will only add to his account that these muscles show not only in their great tendency to this form of contraction, but also in other respects, a great general similarity in their behaviour to that of pressed muscles which Kuehne has so well described. It is often difficult to find a minimal strength of stimulus in the ordinary sense of the expression, the first contraction given by the muscle in response to single induction shocks gradually increasing in strength (e. g., the secondary coil being moved up by half centimetres from a distance of 40 to 30 centimetres from the primary) being a maximal prolonged spasm. Again, if a truly minimal strength of stimulus can be found, a stimulus a very little stronger usually gives rise to a maximal spasm, contractions intermediate in character not being obtainable. A weak stimulus which has elicited such a maximal spasm if repeated may produce no effect whatever, or evoke a comparatively simple twitch somewhat more prolonged in duration than normal. After a maximal spasm has been called forth by a make-shock of moderate strength, the corresponding break-shock may only cause such a twitch, although this merely apparent exception to the general rule of the greater efficacy

* Cf. Biedermann, Electrophysiologie, pp. 366-367, Jena, 1895.

of break-shocks for the production of contracture * is by no means a usual occurrence with the muscles of glycerined frogs.

The spasmodic contractions caused by even strong break-shocks repeated at intervals of one to two minutes vary very much in duration, and often also in amount.

From all this it would seem as though the affection of the muscle which conditions the appearance of contractures were less stable than is the case with veratrinized muscles.

Corresponding with these results it is found that, although ether does away with contracture, normal twitches making their appearance at a certain stage of its action, the cessation of etherization is not usually followed by the reappearance of contractures, as is the rule with veratrinized muscles, and with those treated with 0.6-per-cent sodium-chloride solution. What profound etherization often does for a veratrinized muscle moderate etherization effects for the muscles of glycerined frogs.

The action of ether on the form or contraction of such muscles is well shown in Figs. 7, 8, and 9 (Plates XXIX and XXX).

## IV. Discussion of Results.

After the results obtained from the action of ether in the case of agents so different in their nature as veratrine, physiological salt-solution, and dehydration, there can be but little doubt that, associated with the marked effect of ether on muscular contractility, there is a specific action on contracture in general, contracture disappearing sooner than contractility. How is this to be interpreted?

At the present time, when Gruetzner's doctrine of the composition of most muscles, at any rate, by fibres of two kinds, differing in their characteristic forms of contraction as in other ways, is being generally accepted, one is obliged to regard the action of ether on contracture from this standpoint, especially as Biedermann † has

* Cf. Tiegel, *Arch. f. d. ges. Physiol.*, xiii, 71, 1876; and Locke, *ibid.*, liv, 501, 1893.

† Biedermann, *Wiener Sitzungsber*, xcii (3), 149, 1885; cf. also his Electrophysiologie, p. 93, Jena, 1895.

suggested that an explanation of the dicrotic form of contracture might be found in the composition of ordinary muscles by two kinds of fibres, and Overend,* discussing the subject more recently at some length, regards the alterations of the normal muscle-curve produced by veratrine as being little more than exemplifications of Gruetzner's view, the action of the poison on the "red" slowly acting muscle fibres being supposed to preponderate.

Since, however, the twitch of the "red" fibres, though of longer duration than that of the "white," is not of longer duration (of shorter, in fact, owing to the smaller latent period of the "white" fibres) than is the twitch of the whole muscle, in which both kinds of fibres take part, it is evident that the main problem of contracture—the enormous prolongation of the contracture in response to a single stimulation—remains untouched, whether it be called an affection of the "red" fibres, of the "white" fibres, or of both.†

The question, nevertheless, remains whether a real explanation is afforded by Gruetzner's doctrine of the dicrotism so often found more or less marked in contracture curves.

Awkward at the start are the dicrotic curves in which the notch after the first summit reaches or sinks below the abscissa, the succeeding recontraction, too, sometimes taking place after a distinct interval. Such curves have been noticed by Brunton and Cash, by Mendelssohn, and by Overend himself. To reconcile these with the attempted explanation it seems to me necessary to assume that the inertia of the falling muscle lever and load gained during the relaxation of the quickly acting "white" fibres overcome the contraction of the slowly acting "red" fibres, which must have already commenced unless veratrine,

* Overend, *Arch. f. exp. Path. u. Pharm.*, xxvi, 1, 1890.

† It may be here pointed out that the same consideration holds good in the case of the lengthening of the duration of the simple twitch of a muscle produced by fatigue. Overend (*op. cit.*, p. 17) attempts to explain it by the earlier exhaustion of the "white" fibres. It is, however, obvious that, if this be granted, the *absolute* lengthening in duration of the simple twitch by fatigue—i. e., not merely apparent owing to the lessening of the height of contraction (cf. Marey, Du mouvement dans les fonctions de la vie, p. 340, Paris, 1868; and Volkmann, *Arch. f. d. ges. Physiol.*, iii, 374, 1870)—is as mysterious as ever.

while shortening the latent period of the relaxed whole muscle (Mendelssohn), lengthens enormously the latent period of the relaxed "red" fibres in it.

In most curves of the kind in question the inertia of weight and lever is shown to be at work by the overextension of the muscle in its first relaxation immediately recovered from by its own elasticity (cf. Fig. 10, Plate XXX). Overend's curves, however, obtained with Marey's spring myograph on a drum revolving horizontally, show no signs of such inertia oscillations, and render it probable that a veratrinized muscle can entirely relax after its first twitch before the "after-action" commences. But, if this be so, "red" and "white" fibres fail utterly to explain dicrotism, the more so since Overend himself concluded, from experiments on the rabbit's "red" soleus, that its latent period was shortened by veratrine.

If the dicrotic were the only anomalous form of contracture curve requiring special explanation, one might be tempted, in spite of the difficulties just raised, to still seek to explain it by the existence of two classes of fibres in voluntary muscle. Such is, however, by no means the case, even with veratrine alone. Veratrine curves may possess three well-marked summits, as Overend himself saw, or the second rise and fall of the curves may be broken by a whole series of more or less rhythmical contractions superposed on it. Such irregularities of contracture curves are much more marked in the case of other contracture-producing agents than with veratrine. Fig. 11, Plate XXX, is an example of this in the case of a curarized muscle acted on by physiological salt-solution. A special kind of fibre for each summit in a curve like this can hardly be assumed.

Lastly, the effects of increase of load and of repeated stimulation of the muscle are unfavorable to the application of Gruetzner's doctrine advocated by Overend. The "white" quickly acting fibres are held by all the advocates of the doctrine to be the more easily fatigued, and to be the weaker as far as the lifting of heavy loads is concerned. And yet, as Overend himself found, an increase of the load has more effect in lowering the secondary rise than it has in lowering the initial shortening of a veratrinized muscle—an effect

which I have also observed in the case of muscles acted on by physiological salt solution.* Again, the effect of repeated stimulation in causing the disappearance or marked diminution of all forms of contracture, the ordinary response of the muscle to a single stimulation being usually but little affected, does not harmonize with the view that contracture is especially the function of the fatigue-resisting "red" fibres.

Does now Gruetzner's doctrine afford an explanation of the effect of ether on contracture?

The ease with which it apparently does so is certainly fascinating. One has only to suppose that just as, according to Overend, the action of veratrine is more pronounced on the "red" fibres, the same is true for ether also, the "red" losing their contractility earlier than the "white" fibres. Unfortunately, however, there is in existence experimental evidence which precludes the drawing of this parallel.

It was found by Willy Neumann in Luchsinger's† laboratory, in the case of a great number of substances, including ether, that they uniformly produced their particular effects more speedily upon the more irritable, quickly contracting muscles than upon the less irritable, slowly contracting muscles. The differences in mechanical function between these muscles must be produced, if Gruetzner's doctrine be correct, all the fibres in each muscle not being alike, by the varying predominance of one or other class of fibres in them, and this same varying predominance must have produced the differences in respect to the action of poisons observed by Willy Neumann.‡ It

* Mendelssohn had also found that "le même effet" (diminution of the effect of veratrine on muscle) "est produit par l'augmentation *de la charge*, mais dans ce cas, il n'est pas persistant, et disparait avec la diminution de la charge" (*Comptes rendus de la Soc. de Biol.*, 1883, 150). Since the above was written Schenck (*Arch. f. d. ges. Physiol.*, lxi, 505-508, 1895) has shown that the ratio of height of isometric contraction to height of isotonic contraction is much greater for the "Initialzuckung" than for the "Veratrinverkürzung." "Der Muskel ist in der Initialzuckung weniger dehnbar als in der Veratrinverkürzung."

† Willy Neumann, Ueber toxicologische Verschiedenheiten functionell verschiedener Muskelgruppen. Dissertation, Bern, 1883.

‡ Cf. Gruetzner, *Recueil zoologique Suisse*, i, 678, 1884.

may therefore be concluded that "white," quickly acting fibres are more easily affected by ether than the slowly acting "reds." If, then, there is only to be seen in the action of ether on the veratrinized sartorius a differentiation between "red" and "white" fibres, it must be concluded that the first summit of the dicrotic curve which persists longer under the action of ether must be due to the activity of "red" fibres—a conclusion the reverse of that of Overend, but quite as lacking in any real experimental foundation. Such a conclusion, too, attributes to veratrine the property of reversing the normal contractile relations of the "white" and "red" fibres to one another, the contraction of the "white" becoming slow and prolonged in comparison with that of the "red," which is now relatively the quicker.

It seems to me, therefore, that no such ready explanation as the Gruetznerian doctrine seems at first to afford is available for the action of ether on contracture, and that ether must be regarded as having a specific action on contracture as such, be it of "red" or of "white" muscle fibres.

A second explanation of the action of ether on contracture which offers itself is based upon the assumption that its action on the muscle consists essentially in the change of a strong into a weak stimulus, the latter being unable to produce contracture.

This conception might be upheld in the case of the action of ether on the contracture of muscles treated with salt-solution, since, as a rule, relatively strong stimuli alone cause this form of contraction. It can not, however, hold good in the case of veratrinized muscles, in which minimal stimuli were always found to produce typical contractures, and this even after several strong stimulations sufficiently far apart (Plate XXVII, Figs. 1 and 3).

The action of ether, then, consists in something more than a mere reduction in strength of stimulus as ordinarily understood. While allowing at a certain stage of its action the occurrence of those metabolic processes on which a "twitch" depends, ether prevents the occurrence of those processes on which contracture depends.

What now are the processes on which contracture depends? An

answer to this question has recently been attempted by Schenck,* who supposes the persistent contractions conditioned by ammonia, veratrine, the constant current, spontaneous and heat rigor, and by certain seasonal conditions (Tiegel) to be all due to uncoordinated contractions of the contractile elements of muscle brought about by the inability of these forms of contraction to propagate themselves along the muscle in the normal wavelike manner. It may be possible that the mechanical and other peculiarities of these forms of persistent contractions are, as Schenck supposes, ultimately due to this lack of conductive propagation along muscle fibres, and in spite of the fact that etherization abolishes the conduction of excitation along muscle before it does away with local polar excitability,† it may be possible to reconcile Schenck's view with the fact that etherization suppresses contracture at an earlier period than the normal twitch of the muscle by supposing that, unless the process of excitation be first propagated along the muscle fibre, the local uncoordinated contractions incapable of conduction can not come into existence. What, however, causes these local uncoordinated contractions to follow the passage of a contraction wave through a veratrinized muscle Schenck's theory does not attempt to explain, although the similarity found by him in the mechanical characters of the persistent contractions conditioned by veratrine and by ammonia, the classical chemical stimulus of muscle, is in favour of the probability of a conception which I have long entertained, and which, I think, expresses some, at any rate, of the truth.

With the exception of the unknown seasonal and nutritional conditions which bring about the contracture of Kronecker and Tiegel, I do not know of any agency or substance which conditions contracture in general that is not known to be capable of producing stimulation of muscle itself or of its motor nerve endings. The kind of stimulation in question is that of the agencies described by Biedermann,‡ which "stimulate without killing," in contradistinction to those

* Schenck, *Arch. f. d. ges. Physiol.*, lxi, 494, 1895.

† Biedermann, *Wiener Sitzungsber.*, xcvii (3), 98, 1888.

‡ Biedermann, *Ibid.*, lxxxii (3), 275, 1880.

chemical stimuli of muscle which had been alone recognized by Hermann,* and "locally strongly impair excitability, and, if their action be sufficiently prolonged, destroy it." The former have, so to speak, a chronically stimulating action in contradistinction to the acutely stimulating influence of substances, such as acids and potassium salts, which soon put an end to irritability. May not then the condition of chronic minimal or latent irritation be causally associated with contracture? The increase of excitability caused especially by a strong stimulus,† and known to persist for a considerable time, might make the feeble continuous excitation by the chemical stimulus, which was previously nearly or entirely inefficacious, now capable of marked effect, so that it evokes the various forms of more or less persistent after-contraction. The action of ether is on this hypothesis readily explained, the lowering of excitability caused by it being able to render the feeble more or less latent continuous stimulation incapable of becoming actual long before contractility to strong stimulation is abolished.

Further discussion of the many indirect evidences which favour this view I do not care now to indulge in, but hope to return to the subject later and deal with it in the light of the results of direct experimental evidence.

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## Regarding Positive Kathodic Polarization of Vertebrate Voluntary Muscle.

Although this subject may appear at first sight to be far removed from the action of ether on contracture, it will become evident that the experimental results above described have established a distinct connection between them.

In 1885 Biedermann published experiments on the sartorius of the frog in proof of the existence of positive kathodic polarization of striated muscle.‡ His method was to veratrinize by local applica-

* Hermann, Handbuch der Physiologie, Bd., i (1), p. 105, Leipzig, 1879.

† Cf. Buckmaster, *Arch. f. (Anat. u.) Physiol.*, 1886, 472.

‡ W. Biedermann, *Wiener Sitzungsber.*, xcii (3), 142, 1885.

tion the tibial end only of the frog's sartorius, and then, after an immersion of the whole muscle for from fifteen to thirty minutes in physiological salt solution, to pass through it for a moment from pelvic to veratrinized tibial end the current of two Daniell's cells. Immediately after the breaking of this current the middle of the muscle and its tibial end are connected through a galvanometer, which shows a strong current passing through it from middle to tibial end, due to the local intense and prolonged excitation of the latter due to its veratrinization. This powerful action current is rapidly compensated so as to bring the galvanometer back to zero, and, after first opening the galvanometer circuit, the battery current is again passed through the muscle for one or two seconds in the same descending direction as before. The galvanometer is immediately afterward again connected with the muscle, and now shows a current passing through it from tibial end to muscle middle. The effect of the second passage of the battery current has therefore been to lessen the action current caused by the first passage of it, so that either the middle of the muscle has become more negative or the tibial end more positive. Biedermann held the latter to be the case, and explained the increased positivity of the tibial end as being due to an active inhibitory reaction which occurs at the kathode at break—a reaction opposite in nature to the well-known excitatory action of the kathode at make. Since the galvanometric effect observed indicates a current in the kathodic half of the muscle in the same direction as the battery current which brought it into existence, it was called, in accordance with Hering's nomenclature, a positive kathodic polarization.

Biedermann was able to get similar galvanometric after-effects with unpoisoned muscles, especially after killing their kathodic tibial ends. These effects he similarly interpreted as due to the kathodic muscle substance being left electrically more positive after the passage of a strong constant current of short duration, in consequence of a reaction at break against the kathodal excitation at make.

Working in 1891 in Hering's laboratory, in which Biedermann had also made his experiments, I found that electrical effects analo-

gous to those described by him could be produced by merely stimulating with induction shocks the pelvic end of the sartorius, the tibial end of which had been locally veratrinized in accordance with Biedermann's method.

The induction shocks were led through platinum electrodes close together, and close to the pelvic end of the muscle, the middle and tibial end of which were connected with the galvanometer. The first shock sent into the muscle caused a strong action current, due to the persistent negativity of the veratrinized tibial end. This being rapidly compensated, a second induction shock caused a marked diminution in the strength of this action current, the negativity of the tibial end having been apparently considerably diminished. If, now, Biedermann's method and conclusions were reliable, this effect indicated that an inhibitory after-action of stimulation was conducted to the tonically contracted tibial end along the muscle from the pelvic end—a discovery of some importance, the possibility of which had indeed been referred to by Biedermann himself.

It is obvious, however, that the accuracy of such interpretations of Biedermann's and my own observations depend absolutely on the change of potential indicated by the galvanometer being an increased positivity of the veratrinized tibial end, and not an increased negativity of the middle of the muscle, which would also produce the effect in question.

Following Biedermann's method of poisoning the sartorius, I had, after immersing the tibial end for a few minutes in a dilute veratrine solution, placed the whole muscle for from fifteen to thirty minutes in physiological salt solution. Control experiments now showed that such a muscle, after the destruction of the whole of its veratrinized lower end with a hot glass rod, gave an enormously greater negative variation when stimulated with a single induction shock than can be obtained from an ordinary muscle. Further experiments showed that previous veratrinization was not, as might have been supposed, necessary for the production of this result, but that immersion of the muscle in pure salt-solution was by itself sufficient to produce the effect, which was found myographically to be associated with a contracture-

conditioning action of a 0.6-per-cent solution of sodium chloride, an action analogous to that of veratrine, but not so marked.*

The effects, therefore, which had been observed by Biedermann and myself may have been simply due to greatly increased negativity of the muscle middle, rendered possible by the action of the salt-solution. In fact, in the polarization experiments which I was able to make in control of Biedermann's positive kathodic polarization of normal muscle not locally veratrinized, such a polarization was always much more easily obtained with muscles treated with salt-solution than with muscles not so treated.†

The subject was therefore in need of a thorough revision, which, however, it has not yet received. Biedermann, in his recently published "Electrophysiologie" (pp. 386–391), states merely that he has convinced himself that positive kathodic polarization can occur in normal muscles not locally veratrinized that have not been treated with salt-solution, and gives his old results with locally veratrinized muscles without comment, although, for the reasons already given, they are, in a very high degree, untrustworthy.

It may be here pointed out that experiments made without the use of a normal solution with which the muscle can be kept moist, must be regarded, unless a moist chamber is employed, as by no means free from the possibility of error, inasmuch as the progressive drying of the muscle may condition a prolongation and intensification of excitation analogous to that conditioned by salt-solution.

It is useless too to lay stress, as Biedermann does, on the tendency to a "*polares Versagen*," the inability of currents leaving the muscle through a locally killed or contracted part of it to excite it. There are too many conditions in actual practice tending to prevent this "*polares Versagen*," ‡ for its existence to be assumed in experiments in which it is not demonstrated, and no experiment has hitherto been published in which it was shown that a positive kathodic polarization was unaccompanied by an abnormally intensified contraction of the muscle.

* F. S. Locke, *Arch. f. d. ges. Physiol.*, liv, 501, 1893.
† F. S. Locke, *op. cit.*, p. 517.
‡ Cf. F. S. Locke, *op. cit.*, p. 521.

The question of the action of ether on positive kathodic polarization can now be considered. Biedermann * has himself investigated the effect of etherization on the secondary electromotive properties of muscle, and the comparison of his results obtained with the galvanometer with the myographic observations that have been described in this communication is worth making. Biedermann found that in those cases in which he observed positive kathodic polarization it always disappeared under etherization at a time at which the muscle still twitched in response to the make of the battery current. The three other varieties of secondary electromotive phenomena, not only negative kathodic and positive anodic polarization, which are due to the make kathodic and break anodic excitations respectively, but also negative anodic polarization analogous to which in its inhibitory and assimilatory character Biedermann considers positive kathodic polarization to be—all these persist under complete etherization of the muscle. Such a difference in behaviour can scarcely be explained from Biedermann's standpoint, but is at once comprehensible in the light of the myographic results, if positive kathodic polarization be nothing more than the galvanometric expression of the enormous action currents accompanying contracture.

## Explanation of Muscle Tracings.

### Plates XXVII–XXX.

Large Leclanché cells coupled up in multiple arc two abreast were used to supply the primary coil of the stimulating Du Bois inductorium.

The crosses (× ×) indicate the period of etherization. In the case of curves arranged in "*imbrication latérale*," a cross indicates between which two stimulations etherization was begun or ended. In the case of muscles stimulated surrounded by fluid, a cross indicates when the solution containing ether is turned on or off: in these experiments the curves are, conventionally regarded, inverted.

All curves read from left to right.

### Plate XXVII.

Fig. 1A.—Fresh curarized and veratrinized sartorius stimulated by weak break-shock at commencement of experiment.

* Biedermann, *Wiener Sitzungsber*, xcvii (3), 84, 1888.

Fig. 1B.—The same muscle stimulated every 85.5 to 86.5 seconds with a strong break-shock (distance of secondary from primary, 0 centimetre 2 × 2 Leclanché). Time-marker writes seconds. Load, 80 (÷ 13.5) grammes.

Fig. 2.—Curarized and veratrinized sartorius stimulated every 130.5 to 131.5 seconds with a strong break-shock (distance of secondary coil from primary, 0 centimetre. 2 × 2 Leclanché). Load, 65 (÷ 13.5) grammes. Time-marker writes seconds. This figure is reduced to four fifths of its original size.

Fig. 3A.—Fresh curarized and veratrinized sartorius stimulated with weak break-shock (distance of secondary from primary, 18 centimetres. 2 × 2 Leclanché).

Fig. 3B.—The same muscle stimulated every 81 to 82 seconds with a strong break-shock (distance of secondary from primary, 0 centimetre). Time-marker writes seconds.

Fig. 3C.—The same muscle stimulated, after the completion of Fig. 3B, by a weaker break-shock (distance of secondary from primary, 9 centimetres). Load, 80 (÷ 13.5) grammes.

## Plate XXVIII.

Fig. 4A.—Fresh uncurarized sartorius, surrounded by Ringer's solution, stimulated with strong make- and break-shocks (distance of secondary from primary, 0 centimetre. 4 × 2 Leclanché). Muscle unloaded (i. e., so lightly loaded as just to be able to return to the abscissa). Time-marker writes intervals of three seconds.

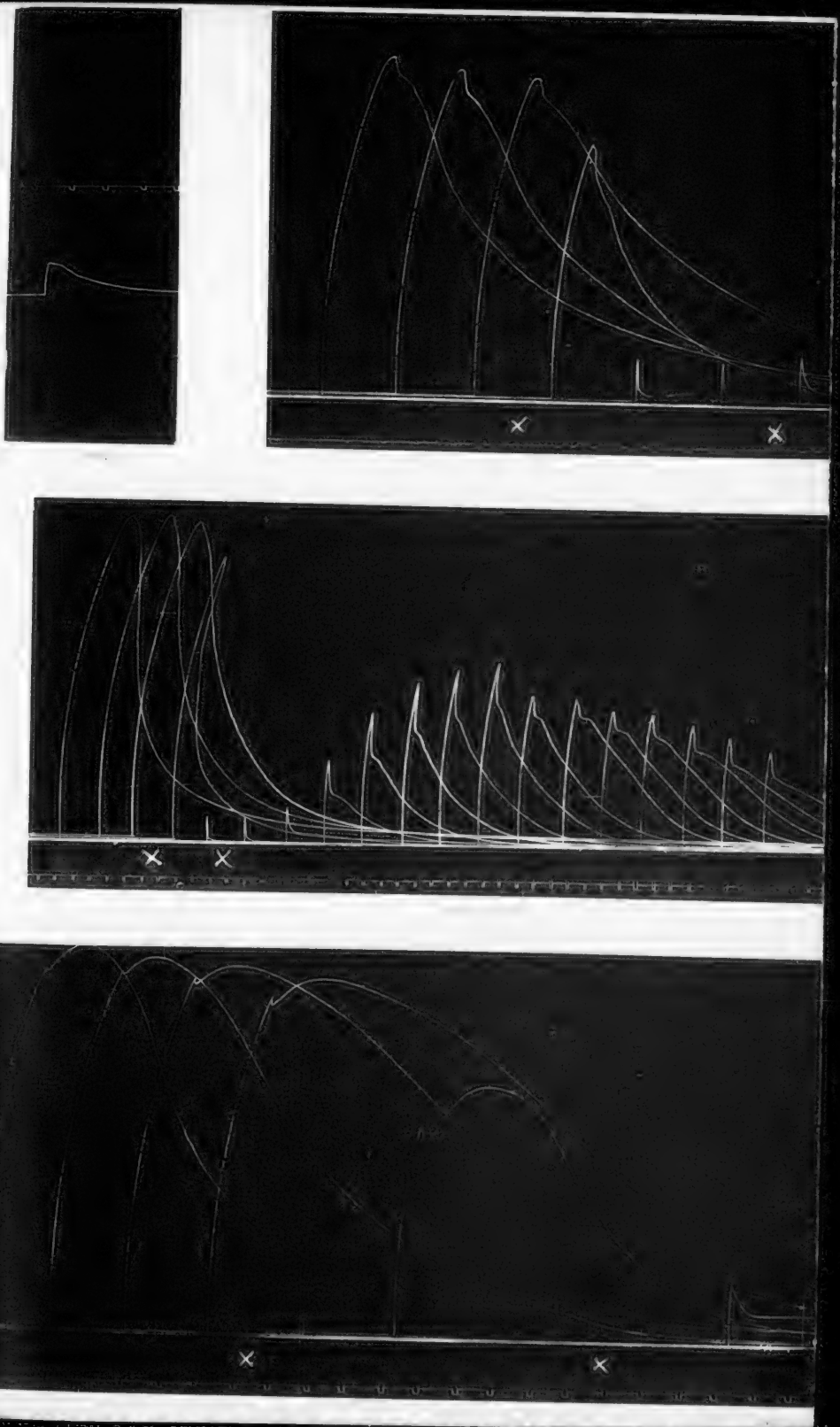
Fig. 4B.—The same muscle, after having been surrounded by 0.6-per-cent NaCl solution for twenty minutes. It is being stimulated every two minutes with a break-shock of the same strength as in Fig. 4A.

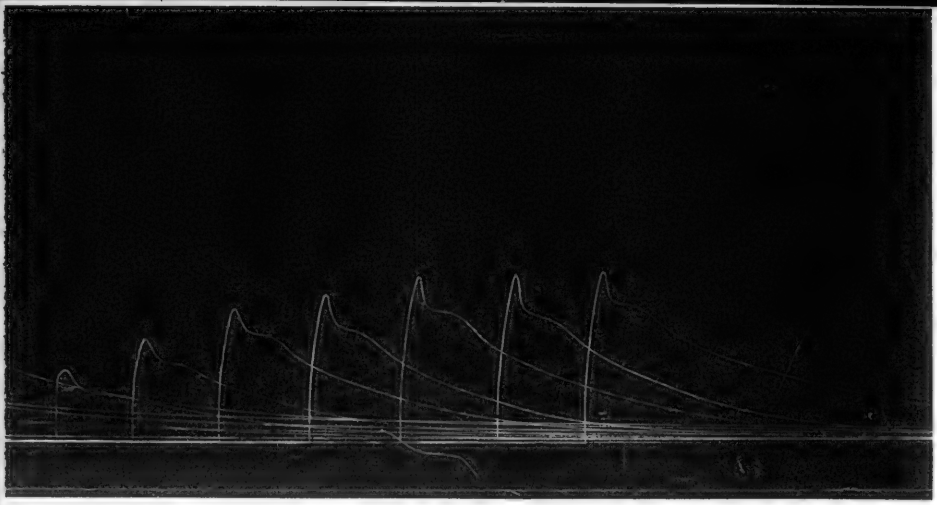
Fig. 4C.—The same muscle at a later stage of the experiment, rhythmical stimulation and the stream of salt-solution having been kept up. The stimulation here took place eight minutes later than the last in Fig. 4B.

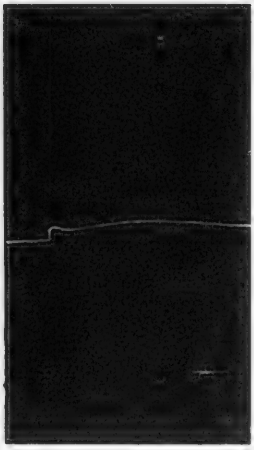
Fig. 5A.—Curarized sartorius in Ringer's solution, containing 0.02-per-cent curare, and stimulated with strong make- and break-shocks (distance of secondary from primary, 0 centimetre. 4 × 2 Leclanché). Muscle unloaded. Time-marker writes intervals of ten seconds.

Fig. 5B.—The same muscle, after having been surrounded with 0.6-per-cent NaCl solution, containing 0.02-per-cent curare, for twenty minutes, without being stimulated electrically, the commencement of rhythmical stimulation (a break-shock of the same strength as in Fig. 5A, every two minutes) being shown here. The temperature in this experiment varied between 15.2° and 15.4° C. Figs. 5A and 5B have been reduced to two thirds of their original size.

Fig. 6.—Curarized sartorius in 0.6-per-cent NaCl solution, containing 0.06-per cent $BaCl_2.2H_2O$ and 0.02-per-cent curare for twenty-three minutes before commencement of etherization, and stimulated eight times at intervals of sixty seconds with a strong break-shock (distance of secondary from primary, 0 centimetre. 5 × 2 Leclanché) before the first similar stimulation shown in the part of the tracing reproduced. Muscle unloaded. Time-marker writes intervals of sixty seconds.







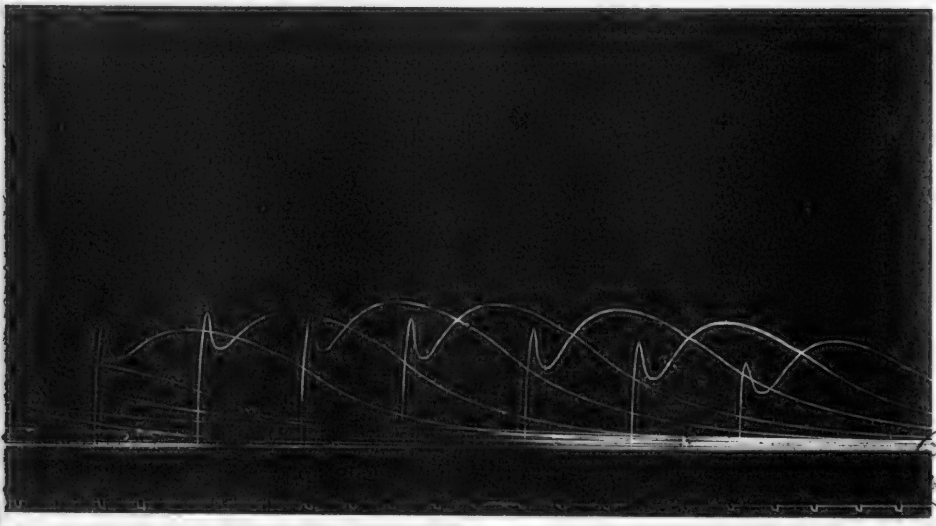


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FIG. 4 A

FIG. 5 A

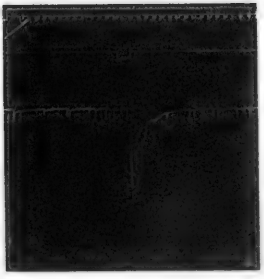

FIG. 4

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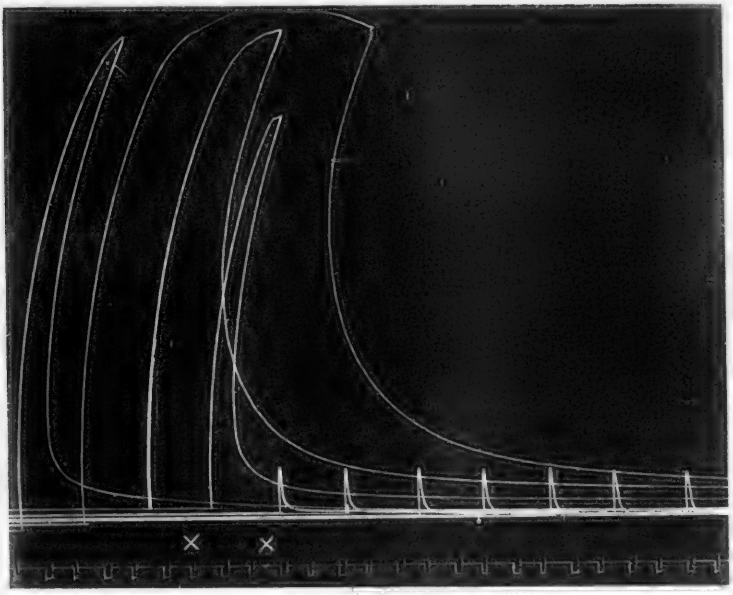

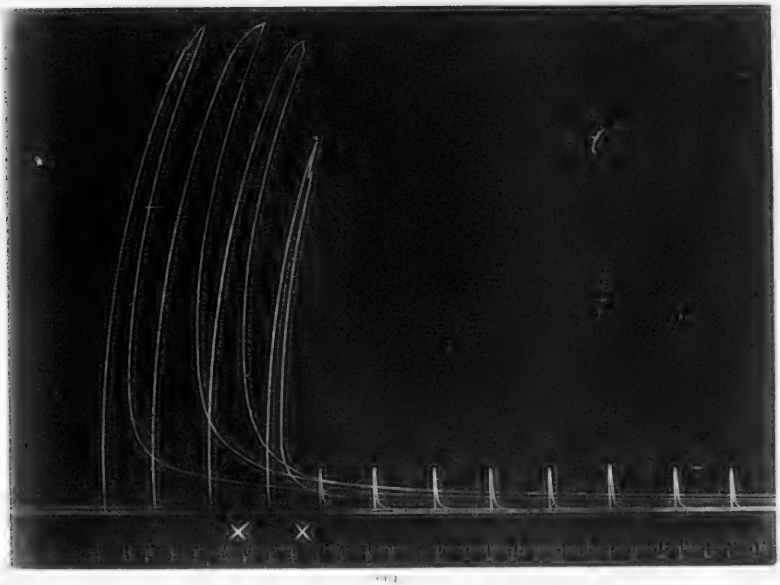

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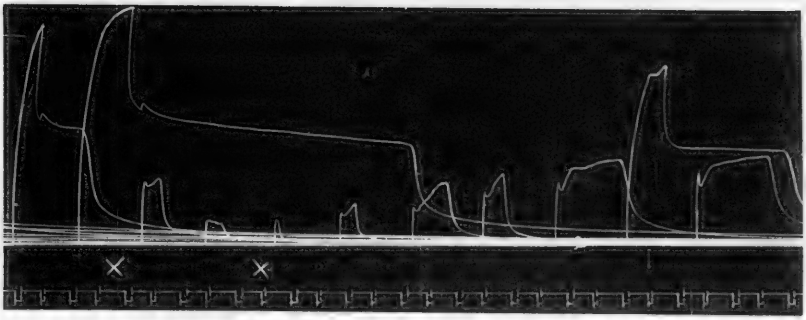

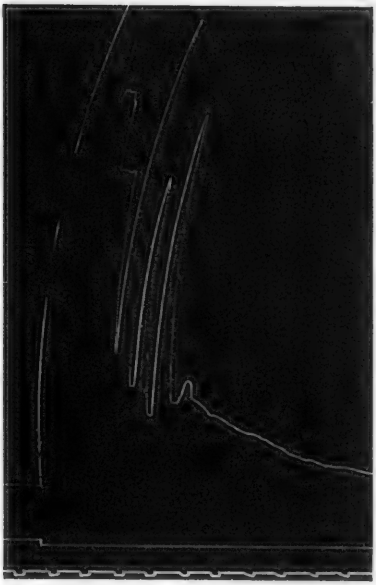

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PLATE XXIX.

Fig. 7.—Curarized sartorius of glycerined frog stimulated every 113.5 to 114.5 seconds by a strong break-shock (distance of secondary from primary, 5 centimetres. 2 × 2 Leclanché). Load, 40 (÷ 13.5) grammes. Time-marker writes seconds.

Fig. 8.—An experiment similar to that of Fig. 7, except that the muscle was stimulated every 137 to 138 seconds.

PLATE XXX.

Fig. 9.—Curarized sartorius of a glycerined frog stimulated with a strong break-shock (distance of secondary from primary, 0 centimetre. 2 × 2 Leclanché) every 120 to 121 seconds. Load, 60 (÷ 13.5) grammes. Time-marker writes seconds.

Fig. 10.—Uncurarized sartorius after an immersion for an unrecorded time in 0.6-per-cent NaCl solution. Poor pale muscle. Load, 20 (÷ 13.5) grammes. Time-marker writes seconds. The muscle had been previously stimulated a few times. The effect of an ascending break-shock (distance of secondary from primary, 0 centimetre) is shown in the figure.

Fig. 11.—Curarized sartorius after an immersion for ten minutes in 0.6-per-cent NaCl solution. Stimulated by an ascending break-shock (distance of secondary from primary, 0 centimetre. 3 × 2 Leclanché). Time-marker writes seconds. The corresponding make-shock had produced a contracture as marked but more regular. Temperature of laboratory and of the salt-solution 21.1° C.

In the case of Figs. 10 and 11 the same muscle lever and moist chamber were used as in the case of Figs. 1, 2, and 3.

# THE REGENERATION OF THE BLOOD.

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AND
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PLATES XXXI, XXXII, AND XXXIII.

THE experiments and observations to be described were made with the twofold object of determining the course of the regeneration of the blood in animals which had been subjected to a loss of a part of the blood and of testing the effect upon blood regeneration of the transfusion of an artificial serum.

The classical observations and experiments of Lyon (1) * and Otto (2) have been the foundation of a large part of the subsequent work in this field. The more exact methods of the present decade open up new problems in the old field and extend its boundaries on all sides. In 1891 Reinert (3) investigated the question of the relation of number of red blood-corpuscles to the amount of hæmoglobin. Last year Koeppe (4) made extended and most careful experiments and observations upon rabbits, from which a part of the blood had been withdrawn. Our experiments differ from Koeppe's in that the withdrawal of blood was followed by an intravenous injection of an artificial serum.

## A. METHODS.

The observations were made upon healthy young dogs of medium size. The experiments upon each animal were introduced with a series of observations upon the normal blood. These were made

* The numbers in parentheses refer to literature at the end of article.

with the hæmatocrit or centrifuge, the hæmometer, the corpuscle-counter, the micrometer, and with the Ehrlich method of staining.

For these observations, the blood was drawn uniformly from the under surface of the ear, which had been prepared by a thorough cleansing with soap and water, with water, and with absolute alcohol. The blood was obtained by making with a sharp, clean lancet a wound large enough to produce in a few seconds a large, free drop of blood.

1. THE METHODS OF OBSERVATION.

*a. The Hæmatocrit.*—1. One end of the capillary tube is placed in the fresh, free drop of blood. Sometimes capillary attraction will fill the tube quickly enough, but usually slight suction must be made with the mouth.

2. The tube is adjusted, the instrument set in motion, and maintained at a steady rate of speed for two minutes, at the end of which time the corpuscles are separated from the plasma and the reading is easily made, as the line between the corpuscles and plasma is distinct.

Hedin (5) suggests that the centrifugation be kept up for at least five minutes; but Hedin's hæmatocrit made only six thousand revolutions per minute, and he always diluted the blood with about equal proportions of an approximately isotonic saline solution. Cowe (7) also dilutes the blood. We do not dilute the blood. Our instrument makes ten thousand revolutions per minute. Repeated trial shows that this rate of rotation will so completely separate the morphotic elements from the plasma that increase of the time to two and a half or three minutes makes no appreciable difference in the reading. The proportion of white corpuscles in normal blood is too small to be even approximately estimated by this method.

*Precautions.*—A free drop of blood must be secured. If the parts are squeezed to get the blood, the proportion of plasma will be increased. Schwyzer (6) also calls attention to this precaution. Not more than thirty seconds should elapse from the time the blood is drawn until it is in the hæmatocrit, for the separation must be prac-

tically complete before coagulation sets in or the observation must be repeated. It is scarcely necessary to say that the capillary tube must be perfectly clean.

*b. The Hæmometer (Fleischl's).*—The purpose of this instrument is to estimate the amount of the hæmoglobin, while the hæmatocrit determines the proportion of red corpuscles to plasma.

The capillary tube which accompanies each instrument is readily filled by capillary attraction on holding one end of the tube to the free drop of blood. The blood is washed with distilled water out of the just filled tube into one chamber of the receptacle, which is then filled level full. The second chamber is also filled with distilled water. The reading is made in a darkened place with the help of artificial light.

*Precautions.*—As with the hæmatocrit, so with the hæmometer, the drop of blood must be free, and not more than thirty seconds should elapse from the time the blood is drawn till it is in the receptacle. The blood should be thoroughly mixed with water. The presence of ever so small a clot necessitates the repetition of the observation. In filling the capillary tube the greatest care should be taken to avoid getting blood upon the outside of the tube and to insure the exact filling of the tube, for a very slight error here would make a difference of ten per cent in the readings. The chambers of the receptacle should not be filled full enough to permit the fluid to mix above the partition.

The most enthusiastic admirer of the hæmometer would not contend that the results obtained with it are more than approximate; they may be correct within about five per cent if the observations were made with all of the enumerated precautions and the readings made with the greatest care. Experimentation has shown that several observers may differ as widely as fifteen per cent on the same reading; furthermore, that the same observer may vary more than ten per cent in repeating a reading. Two simultaneous observations, made upon the same animal, with two Fleischl's hæmometers placed side by side and read by the same individual, may differ as much as eight per cent. Busch and Kerr (8) ascribe to Fleischl's hæmometer

an error range of ten per cent, and to Gower's a still greater inaccuracy.

*c. The Corpuscle Counter (Zeiss's).*—The blood was mixed with an artificial serum made as follows:

| | | |
|---|---|---|
| Gum-arabic solution (sp. gr., 1020)..... | 20 | c. c. |
| Sodium-chloride solution (sp. gr., 1020).... | 30 | " |
| Sodium-sulphate solution (sp. gr., 1020).... | 30 | " |

Mix and filter.

Winternitz (9) advises the dilution of the blood with potassium bichromate (2.5 per cent) for counting the red blood-corpuscles, and with acetic acid (0.1 per cent) for counting the white blood-corpuscles. The above formula advised by Landois answers our purpose well.

*Precautions.*—The dilution of the blood in the "mixer" must be made with the greatest precision, because the microscopical readings are to be multiplied by 400,000. Any error made in the dilution will be multiplied by that factor. The blood must be completely removed from the outside of the end of the mixing tube before dipping it into the artificial serum, otherwise a part of this blood will be drawn into the tube and thus vitiate the results.

When shaking the bulb to effect the complete mixture of the blood with the diluting fluid the rubber tube, which is connected with the upper end of the "mixer," should be drawn tightly across the end of the glass tube to prevent a part of the liquid being thrown out by the centrifugal force. If the mixing tube becomes clogged with a clot, a strong alkali, such as caustic potash (ten to twenty per cent), must be used to dissolve it. Before the tube can be used again it must be freed from every vestige of the alkali, otherwise some of the red blood-corpuscles will be dissolved and disappear.

In cleaning the "mixer" *draw distilled water through it;* if the water be drawn into the bulb, and then forced out by blowing into the rubber tube, saliva may pass into the mixer. In the same way and for the same reasons draw absolute alcohol through the bulb after the latter has been rinsed with distilled water. Dry the tube by *drawing* air through it.

*d. The Micrometer.*—The micrometer is used to determine the diameter of the corpuscles. Many attempt to measure corpuscles which are suspended in a diluted plasma, but Hamburger (10) says that "into whatever solution one may bring the red corpuscles—isotonic, hyperisotonic, or hypisotonic salt or sugar solutions, or diluted serum—the corpuscles always lose the biconcave shape and suffer thereby a lessening of their diameter. If returned to normal plasma, they regain the normal form." After repeated attempts to so adjust our diluting fluid as to lead to no change in the shape of the disks, we adopted the following method, which is very practical, especially for the determination of relative values where absolute values are of secondary importance: Spread upon a cover-glass the thinnest possible film of blood. Exposure to the air will dry it in a few moments. Lay the cover, blood-side down, upon a slide; place under the microscope, and measure corpuscles taken at random from the field, avoiding, however, those which may have become accidentally distorted in the process given above. The average of not fewer than fifty corpuscles should be taken.

The accuracy of the results depends upon the accuracy of the determination of the value of the divisions in the micrometer and upon the accuracy of the measurement of individual corpuscles. Besides the errors of observation incident to the difficulty of getting an absolutely sharp focus of both the corpuscles and the micrometer, and of bringing the micrometer to a point absolutely tangent to the corpuscle—besides these necessary sources of error in observation, there is always a distinct personal error.

*e. Ehrlich's Method of staining Blood, as given by Rieder* (11), *of Munich.*—(1) Place a very small drop of blood upon a perfectly clean cover-glass. Place upon this another cover-glass; press the two together until the blood is in a very thin film; then quickly draw the glasses apart, and expose to the air for a minute or two.

(2) Place the specimens in a drying-oven, and gradually raise the temperature to between 110° C. and 120° C., which temperature is maintained for two hours.

(3) Make an eosin stain according to the following formula:

| | |
|---|---|
| Pure glycerin | 95 c. c. |
| Carbolic acid | 5 " |

Saturate this mixture—or rather 10 cubic centimetres of it—with eosin by stirring the eosin in with a glass rod. To insure saturation there should be an excess of undissolved eosin in the bottom of the receptacle.

(4) Place one or two drops of this saturated eosin-carbol-glycerin upon a slide; invert one of the cover-glass specimens upon the eosin, and leave at room temperature for at least two hours.

(5) Rinse with distilled water.

(6) Treat one minute with hæmatoxylin (Boehmer's or Delafield's).

(7) Rinse again with distilled water, and dry in the air.

(8) Mount in balsam.

*Precautions.*—The cover-glasses used must be absolutely clean. The blood must be spread in a very thin film upon the cover-glass. The staining with hæmatoxylin must be carefully timed.

*General Precaution.*—In all of these different methods of examining the blood three things must be observed: 1. Absolute cleanliness. 2. Exactness in all readings and determinations. 3. Rapidity of work to avoid too early clotting of blood.

### 2. THE WITHDRAWAL OF BLOOD.

*a. Preparation.*—The dog was weighed, fixed to the operating board, and anæsthetized.

The femoral region was chosen as the point for operation, and was prepared according to the rules of aseptic surgery.

*b. Operation.*—An incision is made through the skin over the femoral vessels, whose superficial location facilitates their use for this operation. The artery and vein are dissected to the extent of an inch and a half. A glass cannula, which is provided with a few inches of rubber tubing, is inserted into the artery with the opening toward the heart. The blood flows in jets into a weighed receptacle. A similar cannula, provided with a rubber tube, is inserted into the vein.

This cannula is connected by the rubber tube to a 100-cubic-centimetre burette.

*c. Transfusion.*—Following the American Text-book of Surgery, the word " transfusion " is here used to include the injection into the circulatory system of artificial serum or of normal saline solution. In these experiments we used an artificial serum made after the following formula:

| | |
|---|---|
| Cane sugar | 35 grammes. |
| Common salt | 6 " |
| Sodium hydrate | 0.05 gramme. |

Distilled water, a quantity sufficient to make 1,000 grammes.

The fluid was sterilized by maintaining it at a boiling temperature for three daily periods of one hour each.

The sterilized serum at a temperature of 40° C. was poured into the burette and allowed to flow slowly into the vein under the influence of the hydrostatic pressure. It is needless to say that the necessary precautions were taken to prevent the introduction of air bubbles into the vein. The blood was withdrawn and the fluid injected at the same time. At least thirty minutes should be consumed in the withdrawal of the blood and the transfusion of the fluid, otherwise there will be too rapid a change in the blood pressure, or possibly rather in the quality of the blood, inducing serious symptoms. The amount of blood taken from the dogs was equal to five per cent of the body weight, and the amount of artificial serum injected was equal to six per cent of the body weight, or twenty per cent more than the amount of blood withdrawn.*

*d. After-treatment.*—The wound was closed and aseptically dressed. The dog was kept in a warm room and fed on nourishing broths for three days, after which time he was treated as were the normal dogs.

All of the dogs made a good recovery except one, which had a secondary hæmorrhage on the eighth day, due to his repeated tearing

* In the case of Dog D the amount of blood withdrawn equalled four per cent of the body weight and no transfusion followed.

off of the dressings. In a subsequent operation the vessels of the neck, rather than those of the thigh, were chosen for the operation, with the result that the dressings, being inaccessible to the dog's teeth, were left undisturbed.

## B. Results.

### 1. The tabulated results.

The observations made are given in the following tables.

Table I. Dog A.

| 1 Date. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microme-ter. | 6 Remarks. |
|---|---|---|---|---|---|
| Jan 13th... | 50 | 5·880 | 85 | 5·0μ | |
| " 15th... | 50 | 5·568 | 85 | 5·0μ | |
| " 17th... | 46·6 | 5·304 | 85 | 5·8 | The dog had drunk freely of water about 2 hrs. before observation. |
| **Average....** | **48·9** | **5·586** | **85** | **5·3μ** | |
| Jan. 18th... | 21·6 | 2·490 | 35 | 5·65 | Operation. |
| " 20th... | 22·5 | 2·960 | 40 | 5·45 | Neutrophiles increased. |
| " 22d.... | 23·6 | 1·188 | 45 | 5·45 | " " |
| " 24th... | 26·3 | 1·308 | 47 | 5·2 | |
| **Average....** | **23·5** | **2·238** | **41·7** | **5·4+** | |
| Jan. 27th... | 20 | ..... | 35 | 6·0 | Secondary hæmorrhage. |
| " 29th... | 22·5 | ..... | 40 | 6·0 | Corpuscles very variable in size. |
| " 31st... | 24·2 | ..... | 40 | 6·0 | " " " " |
| **Average....** | **22·5** | **[2·142]** | **38·3** | **6·0** | |
| Feb. 3d..... | 25 | 2·010 | 40 | 6·2 | |
| " 5th.... | 30 | 1·800 | 45 | 6·2 | |
| " 7th.... | 29 | 2·232 | 45 | 6·5 | |
| **Average....** | **28** | **2·014** | **43·3** | **6·3** | |
| Feb. 10th... | 30 | 2·180 | 47 | 6·6 | |
| " 12th... | 33 | 2·228 | 55 | 5·9 | Corpuscles irregular in shape. |
| " 14th... | 35 | 2·160 | 55 | 6·5 | |
| **Average....** | **32·6** | **2·182** | **52·3** | **6·3** | |
| Feb. 17th... | 33 | 3·684 | .... | .... | |
| " 19th... | 34 | 3·440 | 60 | 6·7 | |
| " 21st... | 33 | 4·580 | 60 | 5·7 | |
| **Average....** | **33·3** | **3·901** | **60** | **6·2** | |
| Feb. 24th... | 35 | 3·480 | 75 | 6·2 | |
| " 26th... | 43 | 5·304 | 75 | 6·3 | |
| " 28th... | 45 | 3·216 | 75 | .... | |
| **Average....** | **41** | **3·996** | **75** | **6·25** | Neutrophiles less numerous. |
| March 2d... | 44 | 5·000 | 100 | .... | |
| " 4th.. | 42 | 6·000 | 85 | .... | |
| " 6th.. | 49 | 6·160 | 85 | 6·6 | Corpuscles regular. |
| **Average....** | **45** | **5·720** | **90** | **6·6** | |

Note from Column 1 of Table I that the observations were made upon alternate days, except Sunday, and that the averages usually

represent periods of one week. The date of the operation is recorded in Column 6 under "Remarks." On the day of the operation the observations were made immediately after the withdrawal of blood.

At first thought the observations would seem to be too far apart to show certain important changes which occur the first day after hæmorrhage. Both Otto (2) and Koeppe (4) have recorded and discussed these changes. Their experiments did not involve the intravenous injection of a diluting fluid, and therefore twenty-four to forty-eight hours would be required to so dilute the blood with tissue plasma as to reduce the hæmoglobin and number of red blood-corpuscles to a minimum; in our experiments the injection of an artificial serum reduced the hæmoglobin-content and the number and volume of red blood-corpuscles at once to the minimum, so that the observations taken immediately after the operation always represent the minimum. From this point on the change is one of gradual improvement.

The hæmatocrit observations are recorded in percentages. From this column it will be seen that the average proportion of corpuscles in the normal blood of Dog A is 48.9 per cent, and that immediately after the operation the proportion was reduced to 21.6 per cent. From this point it gradually rises for eight days, when the secondary hæmorrhage brings it back to twenty per cent. From this time the gain, as indicated in the average, is continuous and fairly uniform.

The results obtained with the corpuscle-counter are recorded in Column 3. The observations are recorded in millions. Note an approximate parallelism between the results of the counter and those of the hæmatocrit. That the parallelism is only approximate may be accounted for as follows: The total volume of the corpuscles as observed with the hæmatocrit depends not only upon the number of corpuscles, but also upon the size of the individual corpuscles.

The hiatus in this series of observations (January 27th to 31st) was due to the accidental contamination of the mixing tube and the fluid, used in diluting the blood in the mixer, with the caustic potash, which had been used to clean the mixer. The average here given was interpolated.

The hæmometer readings, which appear in Column 4, are given

on an arbitrary basis of 100, assumed by Fleischl, and approximately representing normal blood. It will be noticed that the results obtained with the hæmometer are closely parallel to those obtained with hæmatocrit and counter. The diameter of the corpuscles is recorded in Column 5 in micromillimetres.

TABLE II. DOG B.

| 1 DATE. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microm-eter. | 6 Remarks. |
|---|---|---|---|---|---|
| Jan. 20th... | 54·8 | 6·280 | 85 | 5·1$\mu$ | |
| " 22d.... | 51·4 | 6·880 | 70 | 5·25 | |
| " 24th... | 53·3 | 6·436 | 95 | 5·25 | |
| **Average....** | **53·1** | **6·532** | **83·3** | **5·17** | |
| Jan. 25th... | 33·3 | ..... | 55 | 5·1 | Operation for withdrawal of blood. |
| " 27th... | 29·7 | 2·796 | 45 | 5·25 | |
| " 29th... | 29·7 | 2·996 | 50 | 5·8 | |
| **Average....** | **30·9** | **2·896** | **50** | **5·5** | |
| Jan. 31st... | 34·5 | 3·480 | 53 | 6·0 | |
| Feb. 5d..... | 23·0 | 1·760 | 40 | 6·5 | |
| " 5th.... | 27 | 2·480 | 45 | 8·0 | Neutrophiles numerous. |
| **Average....** | **28·2** | **2·573** | **49·3** | **6·8** | Corpuscles irregular in shape. |
| Feb. 7th... | 31 | 2·144 | 45 | 6·4 | |
| " 10th... | 33 | 3·560 | 50 | 6·2 | |
| " 12th... | .... | 4·160 | 60 | 6·5 | |
| **Average....** | **32** | **3·288** | **52** | **6·33** | |
| Feb. 14th... | 38 | 3·030 | 60 | 6·5 | |
| " 17th... | 38 | 2·552 | 65 | .... | Neutrophiles. |
| " 19th... | 33·3 | 3·408 | 65 | 5·9 | |
| **Average....** | **36·3** | **2·993** | **63·3** | **6·2** | |
| Feb. 21st... | 36 | 4·688 | 65 | 6·1 | |
| " 24th... | 40 | 7·080 | 90 | 4·6 | |
| " 26th... | 45 | 6·380 | 80 | 6·5 | |
| **Average....** | **40·3** | **6·049** | **78** | **5·7** | |
| Feb. 28th... | 43 | 5·496 | 100 | .... | |
| March 1st... | 50 | 7·880 | 100 | .... | |
| " 4th.. | 49 | 5·000 | 85 | .... | |
| **Average....** | **47·3** | **6·125** | **95** | .... | |
| March 6th... | 54 | 5·288 | 90 | 6·4 | Neutrophiles disappear. |
| " 9th... | 52 | 6·880 | 105 | 6·5 | |
| " 11th.. | 53 | 8·000 | 110 | 6·5 | |
| **Average....** | **53** | **6·722** | **100+** | **6·5** | |

TABLE III. DOG C.

| 1 DATE. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microm-eter. | 6 Remarks. |
|---|---|---|---|---|---|
| April 8th.. | 35 | 5·560 | 70 | 6·7$\mu$ | |
| " 10th.. | 35·6 | 5·290 | 70 | 5·7 | |
| " 13th.. | 37·1 | 5·176 | 60 | 5·85 | |
| **Average....** | **35·9** | **5·342** | **66·6** | **6·1** | |

TABLE III. DOG C—(*Continued*).

| 1 DATE. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microm-eter. | 6 Remarks. |
|---|---|---|---|---|---|
| April 14th.. | 43·6 | 5·000 | 75 | 6·0 | |
| " 16th.. | 40·7 | 5·944 | 70 | 6·5 | |
| " 18th.. | 37·0 | 5·120 | 85 | 5·45 | |
| **Average....** | **40·4** | **5·336** | **76·6** | **6·0** | |
| April 21st.. | 40 | 5·211 | 75 | 6·0 | |
| " 23d... | 43·3 | 5·362 | 80 | 6·0 | |
| " 25th.. | 41·9 | 6·420 | 80 | 6·0 | |
| " 28th.. | 41 | 4·748 | 75 | 6·0 | |
| **Average....** | **41** | **5·435** | **77·5** | **6·0** | |
| May 1st.... | 27·7 | 3·620 | 50 | 5·8 | Withdrawal of blood. |
| " 4th.... | 24 | 4·000 | 50 | 6·0 | |
| " 6th.... | 22·5 | 2·652 | 50 | 6·2 | |
| **Average....** | **24·7** | **3·424** | **50** | **6·0** | |
| May 8th... | 26·0 | 3·216 | 52 | 6·9 | |
| " 10th... | 28·2 | 3·748 | .. | 6·5 | |
| " 12th... | 30·3 | 4·280 | 60 | 6·5 | |
| **Average....** | **26·2** | **3·748** | **56** | **6·6** | |
| May 15th... | 32·5 | 4·990 | 60 | 6·4 | |
| " 19th... | 34·0 | 4·296 | 60 | 6·0 | |
| " 21st... | 40·9 | 5·008 | 75 | 6·6 | |
| **Average....** | **35·8** | **4·764** | **65** | **6·3** | |

TABLE IV. DOG D.

| 1 DATE. | 2 Hæmato-crit. | 3 Counter. (No. in millions.) | 4 Hæmom-eter. | 5 Microm-eter. | 6 Remarks. |
|---|---|---|---|---|---|
| May 4th.... | 46 | 7·000 | 80 | 6·4 | |
| " 6th.... | 40 | 5·000 | 80 | 6·0 | |
| " 8th.... | 40·6 | 5·248 | 80 | 5·7 | |
| **Normal..... | **42·2** | **5·749** | **80** | **6·0** | |
| May 10th... | .... | ..... | .. | ... | Withdrawal of 40 per cent of total volume of blood not followed by transfusion. |
| " 10th... | 32·3 | 5·120 | 70 | 6·0 | |
| " 12th... | 31·0 | 3·964 | 50 | 6·0 | |
| " 14th... | 28·0 | 4·264 | 55 | 6·3 | |
| " 19th... | 30·6 | 4·624 | 70 | 6·5 | |

Tables II and III are similar to Table I, and need no separate explanation. Table IV gives the results of observations on Dog D. This experiment differs from the preceding ones in that the withdrawal of blood was not followed by the transfusion of artificial serum. The record shows that the decrease in the readings after the operation was not so sudden or so great as was the case in the other dogs; further, that the readings continued to decrease for several days, reaching a minimum on the third or fourth day after the opera-

tion, while all of the other dogs reached their minimum immediately after the withdrawal of the blood. This accounts for the apparent retardation of the regeneration of the blood in the case of Dog D.

Dog D was in good condition, and seemed to be perfectly normal during the preliminary observations (May 4th to 10th). His recovery from the loss of blood was not so rapid as was that of the other dogs. The reason for this is apparent; the volume of the blood had been decreased by about one half, and had not been replenished by the transfusion of artificial serum. Four days after the operation the dog showed all the symptoms which follow a severe hæmorrhage, while the other dogs were on the second day quite free from those symptoms.

A comparison of the number of corpuscles in Dog D immediately after the withdrawal of blood with that observed in the other dogs at the same period shows that, while there was a marked falling off in all of the others, in Dog D there was only a slight decrease. A moment's reflection makes it evident that the dilution of the blood with the transfusion fluid will markedly reduce the number of red blood-corpuscles per unit volume. Now, there being, in the case of Dog D, no transfusion of an artificial serum, the blood would be diluted only by the lymph plasma taken up from the tissues. The dilution would naturally be small in amount, as attested by the slight difference, before and after the operation, not only in the number of corpuscles, but also in the volume and in the amount of hæmoglobin.

The fact that our Dog D did not reach a minimum until the fourth day instead of the first or second, as was the case with the rabbits on which Otto and Koeppe experimented, may probably be accounted for by the severer loss which Dog D suffered. Béchamp (12) says that dogs will stand a loss of blood equal to three to four per cent of the body weight; Huenerfauth (13) gives three and a half to four and a half per cent. The limit undoubtedly varies with different individuals.

The symptoms shown by Dog D during the four days subsequent to the operation made it evident that four per cent was practically the limit for that dog. That the fall to minimum should in

this case continue for four days, while in the case where blood equal to only 2.2 per cent of the body weight is withdrawn—see Limbeck's (14) experiment—the minimum should be not much less than the normal and be reached during the first day, is a result to be expected.

In order to get a general view of the results from the three similar experiments on Dogs A, B, and C, the averages from Tables I, II, and III are combined in Table V in such a manner as to make the observations taken immediately after the operation fall in the same column headed "Operation." The observations on Dog C were taken for three weeks before the operation, while on Dogs A and B they were taken for only one week before the operation. This necessitates the interpolation of the averages as given in Columns 4 and 5.

TABLE V. COMBINING THE AVERAGES OF DOGS A, B, C.

| No. | Instruments. | Dog. | Normal I. | Normal II. | Normal III. | Operation 1. | REGENERATION OF THE BLOOD. 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I. | Haemato-crit. | A. | .... | .... | 48·8 | 23·5 | 22·5 | 28·4 | 32·6 | 33·3 | 41 | 45 | ... |
| | | B. | .... | .... | 53·1 | 30·9 | 28·2 | 32·0 | 36·3 | 40·3 | 47 | 56 | ... |
| | | C. | 35·1 | 40·4 | 41·0 | 24·7 | 26·2 | 35·8 | ... | ... | ... | ... | ... |
| | | **Av.** | **[37·1]** | **[16·9]** | **47·6** | **26·3** | **25·6** | **32·1** | **34·4** | **36·8** | **44** | **50** | ... |
| II. | Counter. | A. | .... | .... | 5·58 | 2·24 | [2·14] | 2·01 | 2·18 | 3·90 | 3·99 | 5·72 | 7·19 |
| | | B. | .... | .... | 6·53 | 2·90 | 2·57 | 3·28 | 2·99 | 6·04 | 6·12 | 6·72 | ... |
| | | C. | 5·34 | 5·33 | 5·43 | 3·42 | 3·74 | 4·76 | [4·65] | ... | ... | ... | ... |
| | | **Av.** | **[5·74]** | **[5·73]** | **5·81** | **2·85** | **2·82** | **3·35** | **3·27** | **4·97** | **5·01** | **6·22** | **7·19** |
| III. | Haemom-eter. | A. | .... | .... | 85 | 41·7 | 38·3 | 43·3 | 52·3 | 60 | 75 | 90 | 95 |
| | | B. | .... | .... | 83·3 | 50·0 | 49·3 | 52·0 | 63·3 | 78 | 95 | 100 | 100 |
| | | C. | 66·6 | 76·6 | 77·5 | 50·0 | 56·0 | 65·0 | ... | ... | ... | ... | ... |
| | | **Av.** | **[77·3]** | **[80·9]** | **81·9** | **47·0** | **47·9** | **53·4** | **57·8** | **69** | **85** | **95** | **97** |
| IV. | Micrometer. | A. | .... | .... | 5·2 | 5·4 | 6·0 | 6·3 | 6·3 | 6·2 | 6·2 | 6·6 | 6·5 |
| | | B. | .... | .... | 5·2 | 5·5 | 6·8 | 6·3 | 6·2 | 5·7 | ... | 6·5 | 6·5 |
| | | C. | 6·1 | 5·9+ | 6 0 | 5·9 | 6·6 | 6·3 | ... | ... | ... | ... | ... |
| | | **Av.** | **[5·53]** | **[51·3]** | **5·45** | **5·6** | **6·5** | **6·3** | **6·25** | **6·0** | **6·2** | **6·55** | **6·5** |

This table shows that the regeneration requires several weeks, and that the process is more rapid in the third and the fourth weeks after the operation than in the first and second weeks.

### 2. VARIATIONS OF THE BLOOD WITHIN PHYSIOLOGICAL LIMITS.

By reference to Table III it will be seen that Dog C was under observation for three weeks before the withdrawal of blood, and that

during this time there was quite a variation in the observations made. During the first week there was an increase in the volume and number of the red blood-corpuscles, as shown by the hæmatocrit and counter. The next week the observations vary, but the average for the week is higher than for the previous one. In the third week the observations vary slightly, with an average still higher than that of the second week.

By referring to the weekly averages for the hæmatocrit, counter, and hæmometer, it will be seen that there is a close parallelism in the results of these three methods of observation. But in comparing the daily results they are found to vary widely. It is, of course, possible that this variation may be accounted for on the ground of errors in observation, though the greatest care was taken to reduce such errors to a minimum, as is apparent from the description of methods and precautions. Dogs B, C, and D were taken from the streets, and all seemed to be healthy and in fairly good condition; yet the tables show that they varied greatly as to the quality of the blood. The variation, according to the hæmatocrit, was from thirty-five per cent in Dog C to 54.8 per cent in Dog B. The counter showed 5.56 millions of red blood-corpuscles per cubic millimetre in Dog C and seven millions in Dog D. The hæmometer showed seventy per cent in Dog C and eighty-five per cent in Dog B. Within the limits here indicated the cause and course of the variations are probably physiological. On January 17th, for instance, Dog A drank freely of water. The variations found in the blood on that day would seem to be fully accounted for by the dilution of the plasma, occasioned by the increased imbibition of water. Incident to this dilution there would be relative decrease in the volume of the red blood-corpuscles, relative decrease in the number of corpuscles per unit volume. One would expect also a relative decrease in percentage of hæmoglobin. The latter point was not, however, confirmed. The dilution of the plasma would cause a decrease in its specific gravity, which may account for the increased size of the corpuscles on that day, as shown by the micrometer. But these are only suggestions as to the possible causes.

This subject—*the character, extent, and cause of the variation, within physiological limits, of the quantity and quality of the blood*—is urgently calling for extended investigation. It is the plan of the writers to continue the investigation on the lines marked out in this paper.

The effect of change of altitude has already been investigated by Jaruntowsky and Schroeder (15), Koeppe (16), Siegfried (17), Reinert (18), Grawitz (19), and others; while Czerny (20) and Winternitz (9) have experimented with the changes in the blood induced by change of temperature.

### 3. THE COURSE OF THE REGENERATION OF THE BLOOD.

Regeneration as discussed in the present paper refers especially to the restitution of the whole volume of blood to a quantitatively normal condition. We shall not at present attempt to add to the knowledge of the histogenesis and morphological regeneration of the morphotic elements of the blood—a field already most thoroughly covered by Aquisto (21), Timofejewsky (22), Neumann (23), Vulpian (24), Löwit (25), Engel (26), Zenoni (27), and others.

In order to get a clearer picture of the course of blood regeneration recourse has been had to the graphic method. Attention is invited to Plates XXXI to XXXIII. In these curves the abscissa is divided into six to eleven segments, each representing a period of one week, while the ordinate is divided into ten segments representing the abstract units 1 to 10.

In locating any observation—or rather average—upon these plates the initial digit of the number determines the ordinate of the point, while the time of the observation or average determines the abscissa of the point. Let us take a concrete example: Plate XXXI, Dog A, is derived from the averages in Table I. According to our notation, the first average taken (January 17th) will fall on the first division of the abscissa. The hæmatocrit average—4.89 per cent—will have an ordinate greater than 4 and less than 5—i. e., about 4.9, as nearly as one can locate it on so small a space. In the same way the number of corpuscles per cubic millimetre—5.586 millions—will have an

abscissa of 1 and an ordinate of about 5.6. The averages taken on the 21st of February would all have the abscissa 6, and an ordinate equal respectively to 3.33, 3.9, 6, and 6.2.

Having located the points represented by the averages, one may join these points and thus gain a lucid and correct conception not only of the absolute, but also of the relative changes which the blood underwent. Take, for example, the volume of red blood-corpuscles in Dog A, as represented in the continuous line of Plate XXXI. Note the sudden fall after Period 1, occasioned by the withdrawal of blood. For two periods after the loss of blood there is no essential improvement in the volume of the red blood-corpuscles. With the third period after the withdrawal there begins an increase in the volume of the red blood-corpuscles, which continues, with only minor fluctuations, throughout the remainder of the course of the observations.

Having explained the way in which these curves were traced, and the general interpretation of their rise and fall, we may turn our attention to their special interpretation.

*a. The Relation between Volume of Red Blood-corpuscles and Amount of Hæmoglobin.*—If one compares the hæmatocrit curve—Plate XXXI, Dog A—with the hæmometer curve, one will find that they are practically parallel until Period 5, when they begin to diverge. Inasmuch as this divergence is persistent through four periods and steadily progressive, it requires explanation. The hæmatocrit curve represents *volume* of red blood-corpuscles, while the hæmometer curve represents amount of *hæmoglobin*. The only conclusion that can be drawn is:

*The quantity of hæmoglobin per volume of red blood-corpuscles is not constant.*

*b. The Relation of the Volume of the Red Blood-corpuscles to the Number and Size.*—The volume as recorded by the hæmatocrit varies with the product of two factors: The average volume of the individual corpuscles multiplied by the number of corpuscles per unit volume. This relation may be expressed mathematically by the formula $V \propto v \times n$, in which V equals the total volume. But $v$ can

only be determined through observation of linear dimensions. The diameter only is usually measured.

If all of the dimensions of a red blood disk varied proportionally, then our formula would be $V \propto d^3 \times n$. From this relation we would expect that if the red blood-corpuscles retain their normal volume—i. e., an unvarying diameter, as shown by the micrometer—then the curve representing the number of corpuscles would be practically parallel with the hæmatocrit curve. This is readily shown mathematically by reference to the laws of variables: If $d$ is constant, our formula becomes $V \propto n$, which is equivalent to saying: The curve of Volume is parallel to the curve of Number.

But it is not parallel; it falls below the hæmatocrit curve between the Periods 3 and 5, and after Period 7 rises much above it. This would be mathematically impossible unless there be a variation in the diameter of the corpuscles. And if there is a variation in the diameter of the individual corpuscles, this variation must sustain to the curve of number a reciprocal relation. In other words, if $n$ varies in one direction from V, $d$ must vary in the opposite direction; in the example cited—Plate XXXI, Dog A, Periods 3 to 5—if $n$ becomes relatively less, $d$ should become relatively greater. Now, this phenomenon which the mathematical formula foreshadows is beautifully illustrated and demonstrated in all of the plates. On the other hand, one would expect the micrometer curve to fall after the number curve begins to rise. This may be easily verified in Plate XXXI, Dog B, Plate XXXII, Dog C, and Plate XXXIII, though less easily in Plate XXXI, Dog A, where there is only a slight fall of the micrometer curve in Period 6.

*c. The Relation between the Amount of Hæmoglobin and the Number of Corpuscles.*—That the amount of hæmoglobin does not vary as the volume of the red blood-corpuscles has been demonstrated. A further inspection of the curves shows the hæmometer curves to be practically parallel to their respective number curves. This indicates that *the quantity of hæmoglobin varies as the number of red blood-corpuscles* (Hb $\propto n$), or that each corpuscle has practically the same amount of hæmoglobin, whether it be larger or smaller. One

can easily understand that such would naturally be the case in all variations of size incident to the changes of the specific gravity of the plasma, as was the probable case with Dog A on January 17th.

When, however, the variation is incident to the natural development of a corpuscle, it is more difficult to understand the relations.

Koeppe (4) says that, although it is generally accepted "that in healthy blood the amount of hæmoglobin is, generally speaking, proportional to the number of the red blood-corpuscles," his own observations, as well as those of Reinert (3), would not justify that conclusion. After a careful consideration of the observations of these investigators, the writers feel warranted in reaffirming the tenability of the above very guarded generalization.

Koeppe cites the rapid multiplication of nascent erythrocytes and of microcytes, which increase the number without representing an increase in the Hb. This is unquestionably true, and is demonstrated by the curves on Plate XXXIII, where it will be noted that the number curve is relatively higher during the first three periods after the operation than before or after this time. But this is a special case, and it is not included in the terms of the above formula. Reinert's curves represent single observations and not averages. A large part of the discrepancy between his Hb curves and number curves undoubtedly arises in his failure to neutralize errors of observation by the use of averages.

### 4. THE MICROSCOPICAL OBSERVATIONS.

The well-known observations of Neumann (23), Timofejewsky (22), Zernoni (27), Viola et Jona (28), and others, showing the rapid multiplication of nucleated red blood-corpuscles and of microcytes after hæmorrhage or withdrawal of blood were confirmed in these experiments. There was a moderate increase in eosinophiles, and a very marked increase in neutrophiles during the first week. Besides these confirmatory observations, the experiments afforded a number of suggestive clews, which will be the subject of subsequent experiment.

### 5. On the transfusion of an artificial serum or of a physiological saline solution.

The advantages and disadvantages of this measure as a surgical procedure in cases of severe acute anæmia have been discussed at length by Knoll (29), Ziemssen (31), Feis (32), Ullmann (33), and others. The first classical work in this field was that of Goltz (30), in 1864, who began his experiments upon frogs. He suggested the use of a solution of sodium chloride or some other indifferent solution after severe hæmorrhages, his idea being to avert a profound change in blood pressure. Subsequent experiments show that the change in blood pressure after hæmorrhage is much less marked than had been supposed; it remains true, however, that the regaining of the normal condition and the regeneration of the blood, after a severe hæmorrhage, is much more rapid if there has been a proper intravenous injection. The experiments above described show a very much more rapid recovery of the dogs which were given the benefit of artificial serum transfusion.

### Summary of the Results.

*a.* The blood of apparently normal animals undergoes considerable variations within physiological limits.

*b.* After a loss of blood the regeneration is more rapid if there has been a transfusion of an artificial serum.

*c.* Regeneration after transfusion is less rapid during the first half of the regeneration period than during the second half.

*d.* The regenerative processes once stimulated into activity carry the blood, qualitatively, considerably beyond the established normal. (See Plate XXXIII.) Otto (2) observed a similar phenomenon.

*e.* The quantity of hæmoglobin per volume of red blood-corpuscles is not constant.

*f.* The volume of red blood-corpuscles varies as the product of the average volume of individual corpuscles and the number of corpuscles per unit volume. ($V \propto v \times n$).

*g.* When the number of the corpuscles increases the size decreases, and conversely. ($n \propto \frac{1}{d}$).

*h.* The quantity of hæmoglobin varies in general with the number of red blood-corpuscles per unit volume. (Hb. $\propto n$).

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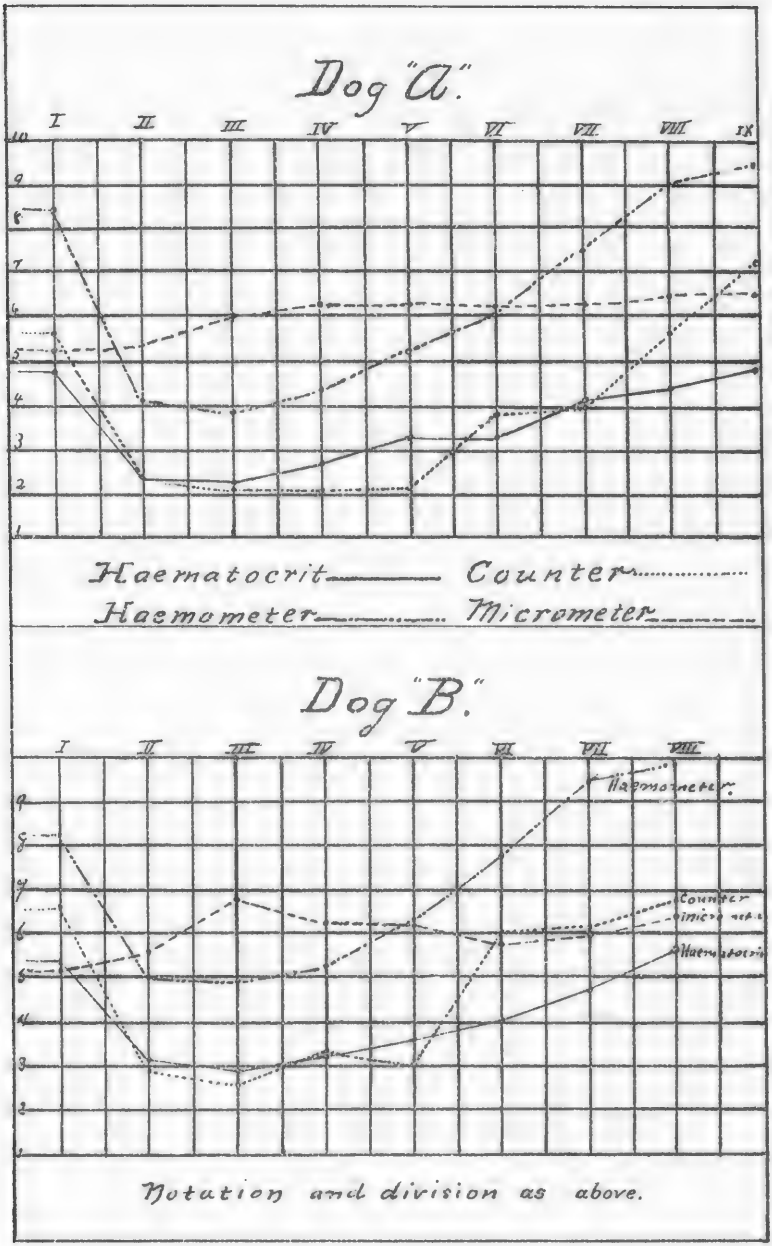
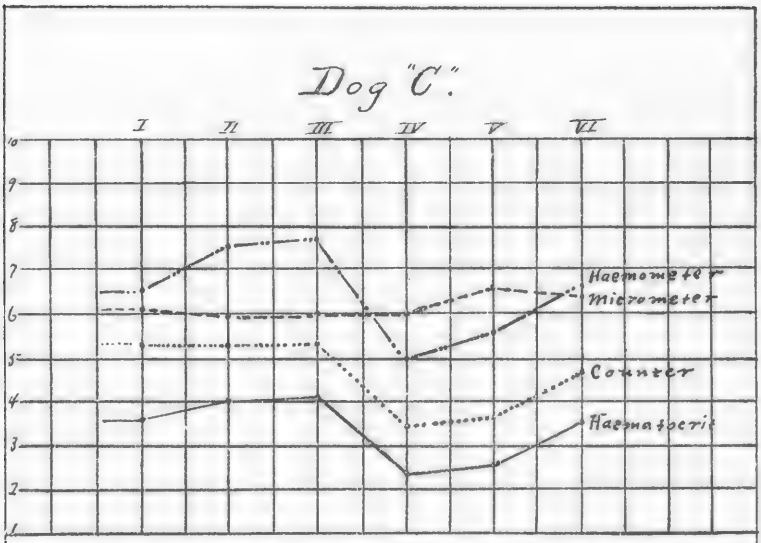
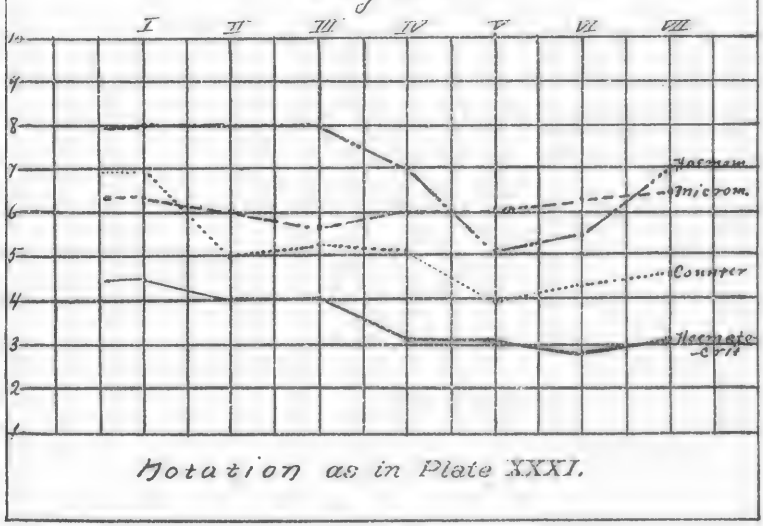
## Description of Plates XXXI, XXXII, and XXXIII.

See the text (pp. 670–672).

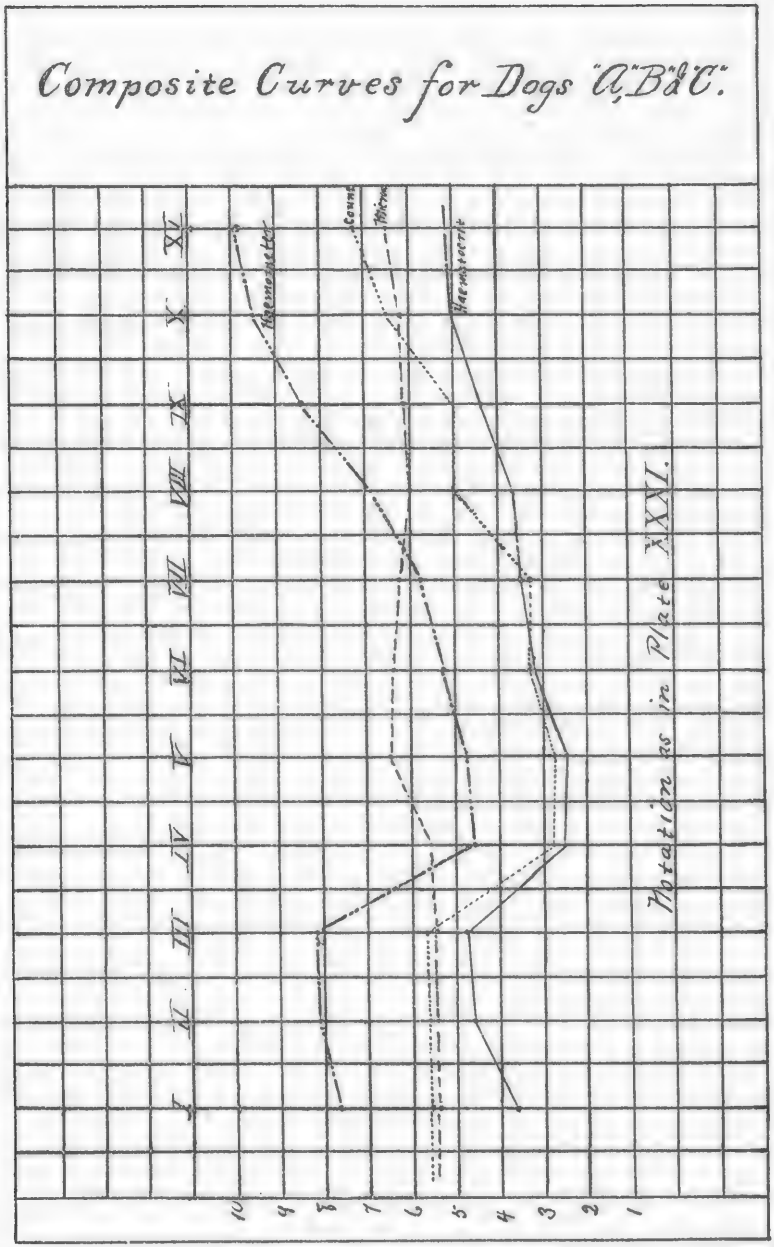

Dog "A."
I
II
III
IV
V
VI
VII
VIII
IX
10
9
8
7
6
5
4
3
2
1
Haematocrit
Counter
Haemometer
Micrometer
Dog "B."
I
II
III
IV
V
VI
VII
VIII
Haemometer.
Counter
Micrometer
Haematocrit
Notation and division as above.




Notation as in Plate XXXI.




Notation as in Plate XXXI.


Composite Curves for Dogs "A," "B" & "C".
Thermometer
Count
Volume
Haemoglobin
Notation as in Plate XXVI.
I
II
III
IV
V
VI
VII
VIII
IX
X
XI
10
9
8
7
6
5
4
3
2
1

# A STUDY OF TYPES OF RESPIRATORY MOVEMENTS.*

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PLATES XXXIV, XXXV, AND XXXVI.

THE relation of the respiratory movements of chest and abdomen has been several times subjected to investigation with diverse results. Boerhaave † called attention to differences in this respect between males and females in 1744. Hutchinson ‡ observed and made drawings of changes in antero-posterior diameters by throwing the shadow of his subject upon a screen. He applied the terms diaphragmatic and costal to types of respiratory movements, and by these terms he meant not only the amount of excursion, as is generally understood, but the time relations. He stated that the respiratory movements begin with the abdomen in men, and that the increase in antero-posterior diameter passes progressively upward, while in women the phenomena are reversed; and, moreover, that all difficult, sudden, and extraordinary breathing is thoracic in this sense. The increase in depth of abdomen in males shown in his drawings seems to be about two or three times that of the chest, while in females it is only one half to two thirds as great. He observed this preponderance of thoracic movement in twenty-four young girls, and was at a loss to account for it, but suggested that it might be a "reservation against the

* Reported to Boston Soc. Med. Sciences, April 17, 1894, and to the Amer. Assoc. for the Advancement of Physical Education, April 5, 1894. This investigation was begun in 1892, while the author held the Bullard Fellowship in the Harvard Medical School.

† Boerhaave, Praelectiones Academicae, vol. v, sect. 623, p. 144. Edit Haller, Amst., 1744.

‡ Todd's Cyclop. Anat. and Phys., article, Thorax.

period of gestation," thus predicating a specific sexual difference. Mays,* from a study of Indian girls, concludes that the difference between the movements of civilized males is due to differences of constriction. He shows tracings in which the abdominal movement is four or five times the thoracic as typical of the normal relation, and adds "the diaphragm has the power of more than doubling the chest capacity, and it is, therefore, the most essential factor of respiration." Kellogg † gives tracings of males and females having the same general appearance as those shown by Mays, and reaches similar conclusions. Wilberforce Smith,‡ on the basis of a series of observations, decides that there is no sexual difference; Foster, Rosenthal, and Carpenter conclude that a sexual difference exists; Waller and Kirk maintain that any observed differences are due to habits of dress.

Ignoring specific sexual differences, there can be no doubt that dress must interfere with the movements of the diaphragm and the lower ribs in those cases where it takes the form of tightly laced corsets, heavy skirts hung from the waist by bands, and belts tightly drawn or loaded with ornaments. This restricting influence of dress is an immediate effect, and may disappear when the tight bands are removed or loosened. On the other hand, permanent reduction of abdominal movement by constriction must be due to a change in the nervous control of the respiratory muscles toward a new co-ordination requiring less muscular effort.# This change of co-ordination forced upon the motor respiratory centres becomes fixed in habit in proportion to the severity and duration of the constriction and the nervous sensibility of the individual. It is impossible to determine the degree of constriction, since the clothing which produces it will

* An Experimental Inquiry into Chest Movements of the Indian Female. *Therapeutic Gazette*, May, 1887. Breathing Movements as a Cure. *Century Magazine*, August, 1893.

† Experimental Researches respecting the Relation of Dress to Pelvic Diseases of Women. J. H. Kellogg, M. D., *Trans. Mich. State Med. Soc.*, 1888.

‡ On the Alleged Differences between Male and Female Respiratory Movements. *Brit. Med. Jour.*, October 11, 1890.

# A preliminary investigation with a belt dynamometer showed that the diaphragm is strengthened rather than weakened by the abdominal resistance offered by corsets, etc.

make difficult the use of recording apparatus, and will render the tracings valueless. For example, a pneumograph over a stiff corset may show no movement while evidences of increased abdominal pressure may be secured by a manometer connected with an air bulb in the rectum (Kellogg). We may take this as evidence that any extra space inside the corset or below it is used in respiration. Again, a pneumograph over folds of thick clothing may not show the real enlargement since part or all of it can be taken up by the clothing. Any record, therefore, with clothing which constricts or cushions the abdomen must be looked upon with suspicion. The immediate interference of dress with the movements of respiration depends so largely upon individual habits in regard to abdominal pressure and the results are so hard to determine for reasons noted above that this study of its influence was limited to the permanent or inhibitive effects, thick clothing and corsets being removed and belts loosened.

In this study simultaneous movements of the thorax and abdomen were recorded by means of belt pneumographs applied over the regions where motion was greatest. No anatomical landmarks were used, for it was soon found that there was considerable individual

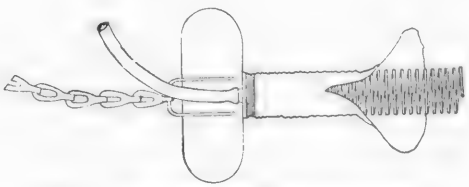


Fig. 1.—Belt-pneumograph, showing structure.

variation in the level of greatest movement. Two pieces of thin rubber tubing, each about fifteen inches long and one inch in diameter, were made into pneumographs (Fig. 1) by the insertion of light coils of wire,* and the addition of a chain by which they were held in place around the body and adjusted for varying circumferences. One end of the tube was closed, the other connected by smaller tubing

* Suggested by Dr. F. W. Ellis.

with a modification of the Ellis piston recorder (Fig. 2), which made tracings corresponding to variations in girth. On account of the length and elasticity of the tubing, the pressure of the pneumograph was too slight to be noticed, all enlargement was faithfully taken up, there was no inelastic resistance and no difficulty or delay in adjusting it to any subject. The calibre of the pneumographs and of the piston recorders was approximately the same, the depth of the tracing was, therefore, the same as the increase in girth.

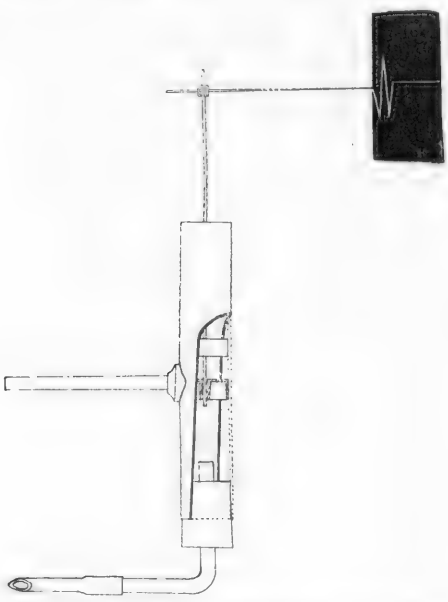


FIG. 2.—Piston recorder, modified for respiratory tracings, and portion of tracing.

A series of calibration tests made upon two subjects to determine the amounts of air moved by chest and abdomen respectively, when acting alone, gave the following results:

| | Normal chest girth. | Normal abdominal girth. | VOLUME OF AIR MOVED BY 10 MM. INCREASE. | |
|---|---|---|---|---|
| | | | Chest. | Abdomen. |
| F. | 101·0 cm. | 87·0 cm. | 780 c. c. | 409 c. c. |
| M | 85·5 " | 71·2 " | 517 " | 357 " |

The volume of air introduced by thoracic movement was to that introduced by the abdominal as 1:0.52 for one subject, and as 1:0.68 for the other.

Five movements of the piston recorders corresponding to the increased girth of thorax and abdomen in five successive respirations

of each individual were measured in millimetres. That part of the tracing was chosen for measurement in which the movements were most regular, and seemed to be most characteristic. To avoid confusion arising from individual variations in depth of respiration, the relation of thoracic to abdominal movement is expressed in the form of a ratio secured by dividing the number representing the chest increase by the number representing the abdominal increase; thus, $\frac{\text{Th } 15}{\text{Ab } 20} = 0.75$. The respiratory ratio is less than unity for all individuals in whom the abdominal movement is greater than the thoracic, and greater than unity in all those in whom the abdominal movement is less. The records are arranged in the tables according to the value of the respiratory ratios $\frac{\text{Th}}{\text{Ab}}$.

The conclusions of this paper are drawn from a study of four hundred and seven respiratory tracings secured from boys and girls, young men and young women, and from individuals of various races found in Chicago during the World's Columbian Exposition.*

Lack of space makes it impossible to publish the complete series of records, but in Tables I to IV are given the individuals of certain groups, selected because they are typical or contribute elements which have been considered factors in determining respiratory types. In each table the records are arranged according to the value of the respiratory ratios, beginning with the lowest. The sums of five consecutive thoracic movements, those of the corresponding abdominal movements and the respiratory ratios are entered in parallel columns with certain other items, as age, childbearing, corset-wearing, etc.

Plates XXXIV to XXXVI † contain distribution curves plotted

* This opportunity is taken to express appreciation of the kind assistance rendered by the managers of various amusement enterprises of the Midway Plaisance, among which may be mentioned the Samoan Village, the Turkish Theatre, the Bedouin Village, the Bedouin Encampment, the Eskimo Village, the Streets of Cairo, the Javanese Village; and also by the manager of a group of Hawaiian dancers which was in Chicago during the summer of 1893, and by Mr. Salisbury, of "Buffalo Bill's Wild West."

† The mean is shown by the apex of the curve; the median, by a heavy vertical line intersecting it.

for each group, the unit of distribution being a difference of 0.5 in respiratory ratio. The curves contain all the records with respiratory ratios less than 4.25 in value, and the percentage of the observations having ratios higher than 4.25 is represented by a vertical line at the end of the curve. As may be seen by reference to Curves I, III, and V, very symmetrical curves of distribution have resulted with only moderate numbers of observations. This leads us to the conclusion that a type of breathing exists which will be true for the majority of any large natural group in spite of individual variations in ratio and depth. Curve VII is an attempt to secure a distribution curve for the respiratory ratios of those females who gave a direct history of constricting dress. It will be seen that there is no mean even with the large unit of distribution. A certain proportion of the ratios fall at various points between 0.0 and 4.25, and, as may be seen by reference to Table III, the ratios above 4.25 (forty-three per cent of the total) were scattered even more irregularly than were those below. We judge, then, that the effect of constriction to the abdomen, not necessarily by corsets and in our subjects very rarely so produced, is to diminish the proportionate movement of the abdomen, and to destroy the natural grouping of the individuals so affected. To show that the unit of distribution chosen is abundantly large, it may be stated that a smooth curve of distribution results in the case of schoolboys and college men with a unit of 0.2 in place of the one of 0.5 which we have used. Curves VI and VIII show the distribution curves of two classes of constricted individuals, Curve VI from the records of twenty-four male Syrians (Bedouins, Turks, etc.) and Curve VIII from the tracings of fifty-two American women, both mixed so far as the effects of constriction are concerned. The abdominal movements of the Syrians had been restricted by tight swathing in childhood and long, tightly wound belts in adult life, while many of the American women had similarly interfered with their respiratory movements by corsets and skirt bands. Certain individuals in both groups were known not to have been seriously constricted, but it was impossible to separate them from the constricted on the evidence available. The curves are flat, a certain proportion of

the ratios occur above the limit and the means are decidedly higher than in Curves I and III. There is a great contrast between these and Curve VII and this would seem to be due to the fact that both constricted and unconstricted individuals are included in the former. The differences between these curves and those for clearly unconstricted groups will be seen in Curve IX on which the curves of schoolboys, college men, constricted males and American women have been plotted for direct comparison. Nearly the same percentages of the four groups have ratios at $\frac{Th}{Ab}$ 1.0, the mean for the two groups under consideration, and we may consider that those percentages corresponding to the portion of the unconstricted groups outside of the lower part of the constricted curves have been pushed into the upper part of the curves and beyond by the constriction. It would seem, therefore, that the occurrence of an apparent mean for the Syrian men and American women at a higher value of the respiratory ratio than for college men is probably due to the combination of respiratory ratios of unconstricted individuals who have naturally a greater relative movement of the thorax than the average, with ratios of others in whom constriction has changed the respiratory co-ordination to a higher level. We are thus forced to regard the means in these curves as having no value in determining the natural relation between thoracic and abdominal movements in respiration.

The remaining curves (II and IV) also show the presence of constriction. The principal evidence in Curve II is the higher level of the mean and the greater breadth of the top of the curve. In Curve IV the mean is at a lower value than in Curve II, but the curve beyond the mean drops much more slowly than in an unconstricted group. A small percentage of ratios in each case falls outside the limits. The absence of notable constriction caused both groups to be regarded as unconstricted, but the analysis of the distribution curves shows that this is not true and proves that even an inelastic string * tied about the waist may modify the respiratory ratio.

*Types of Respiration.*—Respiratory tracings have been published

* Such as worn by the South Sea Island men, etc.

as typical of male respiration in which the abdominal movement was from two to five times as extensive as the thoracic, and as typical of the female type tracings in which the chest movement was correspondingly greater. This "female type" Mays and Kellogg have demonstrated to exist among civilized women only in whom constriction by corsets has effected a change in the natural relation of the movements. These tracings suggested the analysis of the groups into three parts (Table V): Those in whom the abdominal movements were at least double the thoracic; those in whom the thoracic were at least double the abdominal; and those lying between these extremes. In this table the first column shows the total number of observations for the special groups; the second, the percentage of those whose ratios fall below one half; the third, the percentage of those whose ratios fall between one half and two; and the fourth column, the percentage of those whose ratios fall above two. It is interesting to note that the respiratory ratio in the greater percentage of males and females lies between 0.5 and 2.0. Seventy-five per cent of the forty-four females from the World's Fair who gave a history of having worn constricting dress show a thoracic movement two or more times as great as the abdominal. In no other group, however, do we find so large a proportion as this and it is among the Syrian men from whom we got a history of constricting dress that we find the nearest approach to it (forty-two per cent). Among these men, however, fifty per cent of the ratios fall between 0.5 and 2.0. In the last two columns of Table V will be found the percentages of those whose ratios fall below and above unity. Schoolboys, college men and males with unconstricting dress show a distinct tendency to an excess of abdominal movement. Females with constricting dress give a large proportion of thoracic movement; American women a moderate preponderance; schoolgirls, females with unconstricting dress and males with constricting dress have about equal numbers in each group.

*The Male Type.*—In the records of schoolboys and college men we have the best evidence at our disposal of the normal type of male respiration. The mean value of the respiratory ratio for each of these curves occurs at 0.5, the median value for the groups being 0.63 and

0.65 respectively. In the curve of unconstricted males it may be noted that the mean respiratory ratio is at a distinctly higher point than in the curve of American men; but, since these records are not consistent with the others, it seems best to base our conclusions upon results obtained from the larger groups on the ground that the discrepancy probably is due either to the small number of observations (twenty-two) or to slight constriction by waist cords or bands. It appears, therefore, that the normal type of respiration for males is one in which the thoracic movement is between one half and two thirds as great as the abdominal.

*Effect of Constriction.*—The change produced by constriction of the abdomen has been noted in the study of Curves II, VI, VII, and VIII. The apparent effect in any group, every member of which has been subjected to a considerable degree of constriction, is to destroy the normal relation between thoracic and abdominal movements and to cause for each individual an irregular diminution of the abdominal movement in proportion to the extent of the constriction and the sensitiveness of his nervous co-ordination.

*Sexual Differences.*—We have noted evidences of constriction in each of the distribution curves of females. The mean value of the respiratory ratios secured from the Midway females with unconstricting dress (Curve IV) is 0.5, the same as that for schoolboys and college men. In these females the evidence of constriction is to be found in the less rapid fall of the curve above the mean, which raises the median value for the group to 1.12. The mean for schoolgirls and American women is 1.10, the median being 1.02 and 1.25 respectively.

A comparison of the males with constricting dress and the American women is of value since these two groups are practically identical in respect to constriction, each containing individuals whose respiratory movements have been practically unconstricted and others who have been bound. The statement that they have been considered constricted on the basis of inquiry into their mode of dressing, and not from a study of the tracings, may need repetition. Their distribution curves (VI and VIII) and percentages in Table V show the

same characteristics, and this correspondence seems to indicate that the inhibiting influence of dress is in result the same in males and females and, as a corollary, that the natural breathing is the same in both. All the evidence goes to prove that constriction is very effective in changing the relation between thoracic and abdominal movements in respiration, but that sex has played no part in producing the differences in type which have been attributed to it.

*Age.*—In Table I the ages of the schoolboys are given. The larger number of the younger boys is found among the first seventeen of the thirty-five in the group, demonstrating for them a greater proportionate abdominal excursion. A similar predominance may be found in schoolboys as compared with the college men, for sixty-six per cent of the schoolboys are included within the largest group of respiratory ratios, while only fifty-two per cent of the college men fall within the same limits. In Table II we find that the few young children are easily within the first half of the table. These facts may be explained either by the greater tendency in childhood to depend upon ligamentous support of trunk and head and the use of the diaphragm exclusively for breathing, which tendency is lost when the conventional ideas of form are developed resulting in an erect carriage with full muscular support; or by the presence of constriction sufficient to interfere with the respiratory co-ordination.

*Childbearing.*—That childbearing has little permanent effect upon the respiratory ratio may be seen from Tables II and III, in which the records of women who have borne children may be found distributed throughout the tables. Any tendency to thoracic breathing inherited as a "reservation against the period of gestation" would certainly be increased by the repeated occurrence of such condition.

*Correlation.**—In Table VI will be found an attempt to determine the correlation between respiratory ratios and certain physical measurements of groups for which they were obtainable. The numbers which appear in the columns Height, Weight, etc., are the aver-

* Dr. Dudley A. Sargent, Director of the Hemenway Gymnasium of Harvard University, kindly furnished the anthropometric data included in this table.

ages of the measurements of individuals in the groups indicated by the limiting values of $\frac{\text{Th}}{\text{Ab}}$. The numbers in parentheses accompanying these give the number of individuals in each group. Heights, girths and depths are given in centimetres; weight in kilogrammes; capacity of lungs in cubic inches. Total strength is conventional and is obtained by adding together the strength of lungs, back, legs, forearm, and a value for strength of upper arm obtained by multiplying the number of times the person can pull himself up and push himself up into one tenth of his body weight.

The table shows that there is some relation between the respiratory movements and the various measurements. In the college students the chief thing to be noticed is that those who have a ratio $\frac{\text{Th}}{\text{Ab}}$ 0.8 to 1.2 are the largest men in every way. A comparison of the average measurements, lung capacity, girth of chest, etc., with those of the other groups shows that they are related directly as their heights, that is, are large men, and have other measurements in proportion. Of the American women, those who breathe abdominally are heavier, and have correspondingly greater measurements, while those with chest movement in excess are lighter and have smaller measurements. There is here again a suggestion of the physical superiority of the group $\frac{\text{Th}}{\text{Ab}}$ 0.8 to 1.2.

It is not possible to emphasize more strongly the results of this study of correlation because of the large average variation and the small number of observations for analysis, but at some future time more may be attempted in this direction.

## Conclusions.

1. Children of the two sexes differ very little in the character of their respiratory movements.

2. Between girls and women and boys and men there is little or no difference in respiratory type.

3. Childbearing does not permanently affect respiration.

4. The natural type of respiration for both sexes is one in which the movement is fairly equally balanced between chest and abdomen, the abdominal being somewhat in excess.

5. In typical unconstricted individuals the chest contributes about the same bulk of air as does the abdomen.

6. Constricting dress causes preponderance of thoracic movement in ratio to its restriction of abdominal movement and to the sensitiveness of the nervous co-ordination.

Acknowledgments are due to Prof. H. P. Bowditch for suggestions; to Prof. F. W. Putnam and Colonel Bidlake, of the Ethnological Department, World's Columbian Exposition, Dr. J. F. Jones, Dr. Jane D. Kelly, and Miss Helen P. Howell for assistance in securing records; and especially to Mr. G. W. Moorehouse, formerly Assistant in Physiology, for valuable assistance in getting records and in calculating and plotting results.

TABLE I.

*Thirty-five Schoolboys. Average Age, Nine Years Eight Months. Range, Six to Fifteen Years*

| No. | Age. | RESPIRATORY MOVEMENTS IN MM. | | $\frac{Th}{Ab}$ | No. | Age. | RESPIRATORY MOVEMENTS IN MM. | | $\frac{Th}{Ab}$ |
|---|---|---|---|---|---|---|---|---|---|
| | | Thorax. | Abdomen. | | | | Thorax. | Abdomen. | |
| 1 | 8 | 5·0 | 19·0 | 0·26 | 19 | 12 | 15·0 | 25·0 | 0·60 |
| 2 | 7 | 15·0 | 52·0 | 0·29 | 20 | 10 | 11·0 | 17·0 | 0·65 |
| 3 | 6 | 7·0 | 14·0 | 0·29 | 21 | 12 | 29·0 | 44·0 | 0·66 |
| 4 | 9 | 7·0 | 20·0 | 0·35 | 22 | 9 | 29·0 | 43·0 | 0·68 |
| 5 | 7 | 10·0 | 27·0 | 0·37 | 23 | .. | 31·0 | 45·0 | 0·69 |
| 6 | .. | 10·0 | 27·0 | 0·37 | 24 | 6 | 18·0 | 22·0 | 0·82 |
| 7 | 7 | 9·0 | 22·0 | 0·41 | 25 | 10 | 14·0 | 16·0 | 0·88 |
| 8 | 12 | 9·0 | 21·0 | 0·43 | 26 | 7 | 15·0 | 17·0 | 0·88 |
| 9 | 7 | 13·0 | 30·0 | 0·43 | 27 | 14 | 28·0 | 31·0 | 0·90 |
| 10 | 8 | 9·0 | 24·0 | 0·47 | 28 | 6 | 16·0 | 17·0 | 0·94 |
| 11 | 6 | 12·0 | 23·0 | 0·52 | 29 | 11 | 24·0 | 24·0 | 1·00 |
| 12 | 10 | 11·0 | 21·0 | 0·52 | 30 | 14 | 30·0 | 29·0 | 1·04 |
| 13 | 14 | 13·0 | 24·0 | 0·54 | 31 | .. | 71·0 | 66·0 | 1·08 |
| 14 | 8 | 12·0 | 22·0 | 0·55 | 32 | 15 | 33·0 | 28·0 | 1·18 |
| 15 | 9 | 11·0 | 20·0 | 0·55 | 33 | 11 | 27·0 | 12·0 | 2·25 |
| 16 | 13 | 15·0 | 27·0 | 0·56 | 34 | 10 | 66·0 | 23·0 | 2·87 |
| 17 | 8 | 12·0 | 21·0 | 0·57 | 35 | 12 | 66·0 | 7·0 | 9·43 |
| 18 | .. | 12·5 | 21·0 | 0·60 | | | | | |

TABLE II.

*Thirty-four Females. Unconstricting Dress. (Midway Plaisance.)*

| Number. | Race. | Age. | Children. | RESPIRATORY MOVEMENTS IN MM. | | $\frac{Th}{Ab}$ |
|---|---|---|---|---|---|---|
| | | | | Thorax. | Abdomen. | |
| 1 | Es. | 4 | .. | 0·0 | 7·0 | 0·00 |
| 2 | Es. | 3 | .. | 2·0 | 11·5 | 0·17 |
| 3 | Es. | 5 | .. | 4·5 | 18·0 | 0·25 |
| 4 | Sy. | 45 | 6 | 4·0 | 10·8 | 0·37 |
| 5 | I. | 15 | .. | 8·0 | 20·0 | 0·40 |
| 6 | Es. | 7 | .. | 4·0 | 10·0 | 0·40 |
| 7 | I. | 8 | .. | 5·0 | 11·8 | 0·43 |
| 8 | H. | 24 | 2 | 10·0 | 22·5 | 0·45 |
| 9 | Sa. | 19 | .. | 9·0 | 18·6 | 0·48 |
| 10 | I. | 11 | .. | 9·0 | 16·0 | 0·56 |
| 11 | I. | 26 | 3 | 15·0 | 23·0 | 0·65 |
| 12 | H. | 21 | .. | 20·3 | 29·0 | 0·72 |
| 13 | J. | 18 | 1 | 9·0 | 12·0 | 0·75 |
| 14 | Sa. | .. | .. | 7·0 | 9·3 | 0·75 |
| 15 | I. | 50 | 3 | 11·0 | 14·5 | 0·76 |
| 16 | Eg. | 15 | .. | 8·5 | 11·0 | 0·77 |
| 17 | Es. | 18 | .. | 12·0 | 13·0 | 0·93 |
| 18 | H. | 19 | .. | 12·2 | 12·0 | 1·02 |
| 19 | J. | 17 | .. | 16·0 | 13·0 | 1·23 |
| 20 | I. | 60 | 9 | 8·3 | 6·5 | 1·28 |
| 21 | I. | 20 | .. | 16·5 | 12·0 | 1·38 |
| 22 | Es. | 55 | 2 | 14·0 | 10·0 | 1·40 |
| 23 | Sa. | 17 | .. | 12·0 | 8·6 | 1·40 |
| 24 | I. | 18 | .. | 15·0 | 10·0 | 1·50 |
| 25 | H. | 20 | .. | 17·0 | 11·0 | 1·54 |
| 26 | I. | 21 | 4 | 22·5 | 12·6 | 1·79 |
| 27 | Es. | 39 | .. | 24·0 | 13·0 | 1·85 |
| 28 | Sa. | 15 | .. | 10·0 | 5·3 | 1·89 |
| 29 | Es. | 40 | 11 | 15·0 | 7·8 | 1·94 |
| 30 | Es. | 14 | .. | 12·0 | 5·0 | 2·40 |
| 31 | I. | 28 | .. | 20·5 | 7·0 | 2·93 |
| 32 | Sy. | 26 | .. | 6·0 | 2·0 | 3·00 |
| 33 | Es. | 13 | .. | 20·5 | 6·0 | 3·41 |
| 34 | I. | 30 | 2 | 24·5 | 3·0 | 8·17 |

Es. = Eskimo. Eg. = Egyptian. Sy. = Syrian. I. = American Indian.
Sa. = Samoan. H. = Hawaiian. J. = Javanese.

TABLE III.

*Forty-four Females. Constricting Dress. (Midway Plaisance.)*

| Number. | Race. | Age. | Children. | RESPIRATORY MOVEMENTS IN MM. | | Th Ab |
|---|---|---|---|---|---|---|
| | | | | Thorax. | Abdomen. | |
| 1 | Eg. | 20 | .. | 5·6 | 11·5 | 0·48 |
| 2 | Eg. | 15 | .. | 11·0 | 10·0 | 1·10 |
| 3 | Es. | 30 | 8 | 10·0 | 9·0 | 1·11 |
| 4 | Eg. | 17 | .. | 5·0 | 4·0 | 1·25 |
| 5 | Sy. | 32 | .. | 17·0 | 12·5 | 1·36 |
| 6 | Eg. | 23 | .. | 11·6 | 7·6 | 1·53 |
| 7 | Es. | 45 | 13 | 11·0 | 7·0 | 1·60 |
| 8 | Sy. | 16 | .. | 8·5 | 5·0 | 1·70 |
| 9 | Es. | 17 | .. | 21·0 | 12·3 | 1·71 |
| 10 | J. | 15 | .. | 17·0 | 8·0 | 2 13 |
| 11 | Sy. | 28 | 3 | 15·0 | 7·0 | 2·13 |
| 12 | Sy. | 21 | 2 | 11·5 | 5·0 | 2·30 |
| 13 | Es. | 18 | .. | 16·0 | 7·0 | 2·30 |
| 14 | Es. | 16 | .. | 14·0 | 6·0 | 2·33 |
| 15 | Sy. | 35 | .. | 12·0 | 5·0 | 2·40 |
| 16 | Sy. | 13 | .. | 13·0 | 5·0 | 2·60 |
| 17 | I. | 31 | 1 | 21·0 | 8·0 | 2·62 |
| 18 | Es. | 16 | 1 | 11·0 | 4·0 | 2·75 |
| 19 | Es. | 17 | .. | 16·0 | 5·0 | 3·20 |
| 20 | Eg. | 25 | .. | 20·0 | 6·0 | 3·33 |
| 21 | Es. | 35 | 3 | 10·3 | 3·0 | 3·43 |
| 22 | J. | 18 | 1 | 7·3 | 2·0 | 3·65 |
| 23 | J. | 20 | .. | 8·0 | 2·0 | 4·00 |
| 24 | Es. | 14 | .. | 25·0 | 6·0 | 4·17 |
| 25 | Sy. | 32 | 2 | 8·5 | 2·0 | 4·25 |
| 26 | Es. | 24 | .. | 23·0 | 5·0 | 4·60 |
| 27 | Es. | 28 | .. | 14·2 | 3·0 | 4·73 |
| 28 | Sy. | 40 | 4 | 24·0 | 5·0 | 4·80 |
| 29 | Eg. | 25 | .. | 10·0 | 2·0 | 5·00 |
| 30 | Sy. | 21 | .. | 31·0 | 6·0 | 5·16 |
| 31 | J. | 30 | .. | 11·0 | 2·0 | 5·50 |
| 32 | Sy. | 18 | .. | 11·0 | 2·0 | 5·50 |
| 33 | Eg. | 10 | .. | 16·0 | 2·0 | 8·00 |
| 34 | J. | 48 | 4 | 16·6 | 2·0 | 8·30 |
| 35 | J. | .. | 1 | 22·0 | 2·0 | 11·00 |
| 36 | I. | 45 | 1 | 33·3 | 3·0 | 11·10 |
| 37 | Sy. | 17 | .. | 25·0 | 2·0 | 12·50 |
| 38 | Sy. | 40 | 8 | 35·5 | 2·0 | 17·75 |
| 39 | J. | .. | .. | 12·0 | .... | ∞ |
| 40 | J. | 23 | .. | 12·3 | .... | ∞ |
| 41 | I. | 42 | .. | 13·5 | .... | ∞ |
| 42 | J. | 30 | 1 | 15·0 | .... | ∞ |
| 43 | Sy. | 16 | .. | 15·0 | .... | ∞ |
| 44 | J. | .. | .. | 16·5 | .... | ∞ |

Eg. = Egyptian. Es. = Eskimo. Sy. = Syrian. J. = Javanese. I. = American Indian.

TABLE IV.

*Fifty-two American Women. Average Age, Twenty-two Years Five Months. Range, Seventeen to Thirty-four Years.*

| No. | Age. | Corset. | RESPIRATORY MOVEM'TS IN MM. | | Th / Ab | No. | Age. | Corset. | RESPIRATORY MOVEM'TS IN MM. | | Th / Ab |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Thorax. | Abdomen. | | | | | Thorax. | Abdomen. | |
| 1 | 21 | No. | 2·0 | 19·0 | 0·11 | 27 | 20 | No. | 35·0 | 28·0 | 1·25 |
| 2 | 17 | No. | 6·0 | 14·0 | 0·43 | 28 | 19 | 3 (2) | 55·0 | 44·0 | 1·25 |
| 3 | 19 | 4 | 15·5 | 36·0 | 0·43 | 29 | 18 | Yes. | 23·0 | 17·3 | 1·33 |
| 4 | 19 | 1½ | 14·0 | 30·0 | 0·47 | 30 | 25 | Yes. | 39·0 | 29·0 | 1·35 |
| 5 | 24 | Yes. | 11·0 | 22·5 | 0·49 | 31 | 25 | No. | 18·0 | 13·3 | 1·36 |
| 6 | 19 | No. | 21·0 | 40·0 | 0·53 | 32 | 34 | No. | 46·0 | 29·0 | 1·59 |
| 7 | 21 | W. | 37·5 | 62·7 | 0·60 | 33 | 20 | No. | 16·0 | 10·0 | 1·60 |
| 8 | 26 | 11 | 22·0 | 33·0 | 0·67 | 34 | 31 | No. | 24·5 | 15·0 | 1·63 |
| 9 | .. | .... | 15·7 | 22·0 | 0·71 | 35 | 25 | No. | 27·0 | 16·0 | 1·69 |
| 10 | 25 | No. | 27·5 | 37·7 | 0·73 | 36 | .. | No. | 28·7 | 16·0 | 1·79 |
| 11 | 22 | 9 (3) | 26·0 | 31·7 | 0·82 | 37 | 22 | No. | 23·0 | 11·0 | 2·10 |
| 12 | 28 | No. | 12·0 | 14·3 | 0·84 | 38 | .. | .... | 25·0 | 11·0 | 2·28 |
| 13 | 23 | 3 (2) | 19·3 | 21·3 | 0·91 | 39 | 30 | Yes. (3) | 16·0 | 7·0 | 2·29 |
| 14 | 24 | No. | 55·0 | 59·0 | 0·93 | 40 | .. | .... | 61·0 | 25·5 | 2·39 |
| 15 | 24 | No. | 20·0 | 21·0 | 0·95 | 41 | 25 | 9 | 75·0 | 25·0 | 3·00 |
| 16 | 21 | W. | 23·0 | 23·3 | 0·99 | 42 | 20 | No. | 47·0 | 15·0 | 3·13 |
| 17 | 25 | No. | 13·7 | 13·3 | 1·03 | 43 | 18 | 2 (2) | 15·7 | 5·0 | 3·14 |
| 18 | .. | .... | 14·7 | 14·0 | 1·05 | 44 | .. | .... | 26·0 | 5·0 | 5·20 |
| 19 | 24 | Yes. | 8·0 | 7·5 | 1·07 | 45 | 19 | No. | 54·7 | 9·0 | 6·08 |
| 20 | 19 | 1 (2) | 17·8 | 16·0 | 1·11 | 46 | 17 | No. | 38·0 | 6·0 | 6·33 |
| 21 | 20 | No. | 27·0 | 23·7 | 1·14 | 47 | 28 | 3 (3) | 74·0 | 11·0 | 6·73 |
| 22 | 18 | 3 | 19·0 | 16·0 | 1·19 | 48 | 28 | 1 (6) | 95·0 | 14·0 | 6·80 |
| 23 | 21 | No. | 21·7 | 18·3 | 1·19 | 49 | 17 | Yes. | 77·0 | 10·0 | 7·70 |
| 24 | 20 | Yes (2) | 31·5 | 26·3 | 1·20 | 50 | 17 | 2 | 19·0 | .... | ∞ |
| 25 | 25 | 12 | 22·5 | 18·7 | 1·21 | 51 | 20 | No. | 23·0 | .... | ∞ |
| 26 | 32 | 12 | 21·0 | 17·0 | 1·24 | 52 | 25 | No. | 66·0 | .... | ∞ |

Yes = Corset worn, length of time not stated. 4 = Corset worn for four years. (3) = Discontinued wearing corsets three years ago. W = Corset waists worn. No = Never wore corsets.

TABLE V.

*Percentage Distribution of Observations in relation to* $\frac{Th}{Ab}$

| GROUP. | No. of observations. | RATIOS. | | | | |
|---|---|---|---|---|---|---|
| | | 0·0 to 0·5. | 0·5 to 2·0. | 2·0 to ∞. | 0·0 to 1·01. | 1·0 to ∞. |
| Schoolboys | 35 | 28·6 | 62·8 | 8·6 | 82·8 | 17·2 |
| Schoolgirls | 72 | 1·4 | 83·3 | 15·3 | 52·7 | 47·3 |
| College men | 124 | 27·4 | 66·9 | 5·7 | 78·0 | 22·0 |
| Females unconstricted | 34 | 26·5 | 58·7 | 14·8 | 50·0 | 50·0 |
| Males unconstricted | 22 | 13·7 | 81·7 | 4·6 | 72·8 | 27·2 |
| Males constricted | 24 | 8·3 | 50·0 | 41·7 | 41·6 | 58·4 |
| Females constricted | 44 | 2·3 | 22·6 | 75·1 | 2·3 | 97·7 |
| American women | 52 | 9·6 | 59·6 | 30·8 | 30·8 | 69·2 |

TABLE VI.

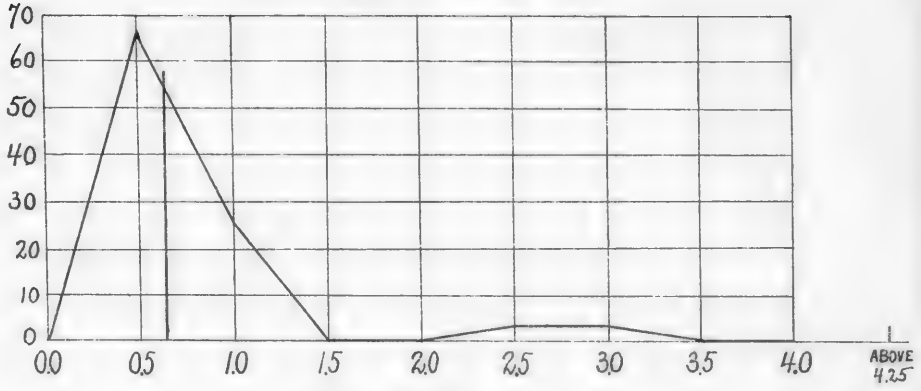
*Correlation.*

| | Th Ab | Height. | Weight. | Capacity of lungs. | Total strength. | CHEST NORMAL. | | | ABD. NORMAL. | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | Chest at rest. | Increase in respiration. | Depth. | Girth. | Depth. |
| College men. | 0·0–0·4 | 173·0 | 64·3 | 256·0 | 493·0 | 87·6 | 5·03 | 18·7 | 73·5 | 18·3 |
| | | (15) | (15) | (14) | (14) | (15) | (15) | (15) | (15) | (15) |
| | 0·4–0·8 | 172·7 | 62·4 | 254·0 | 468·3 | 86·1 | 5·25 | 18·9 | 72·2 | 18·3 |
| | | (40) | (40) | (39) | (39) | (40) | (40) | (40) | (40) | (40) |
| | 0·8–1·2 | 175·2 | 65·3 | 270·5 | 525·9 | 88·2 | 5·2 | 20·4 | 68·7 | 18·6 |
| | | (13) | (13) | (13) | (13) | (13) | (13) | (13) | (13) | (13) |
| | 1·2–2·0 | 174·2 | 61·4 | 245·3 | 470·4 | 85·5 | 5·2 | 18·5 | 71·0 | 18·0 |
| | | (15) | (15) | (15) | (15) | (12) | (15) | (15) | (15) | (15) |
| | 2·0–4·0 | 173·0 | 59·0 | 258·3 | 473·7 | 83·1 | 5·9 | 18·4 | 69·9 | 18·0 |
| | | (6) | (6) | (6) | (6) | (6) | 6) | (5) | (5) | (5) |
| American women. | 0·0–0·8 | 160·3 | 57·4 | 152·0 | ..... | 80·6 | 5·3 | 17·3 | 64·3 | 18·8 |
| | | (6) | (6) | (5) | | (5) | (5) | (5) | (5) | (5) |
| | 0·8–1·2 | 161·0 | 55·2 | 166·6 | ..... | 78·2 | 5·5 | 17·3 | 62·6 | 16·5 |
| | | (5) | (5) | (5) | | (5) | (5) | (5) | (5) | (5) |
| | 1·2–6·0 | 160·0 | 50·5 | 139·0 | ..... | 77·7 | 4·3 | 15·7 | 59·8 | 15·0 |
| | | (5) | (5) | (5) | | (5) | (5) | (4) | (5) | (4) |
| | 6·0+ | 160·6 | 46·6 | 130·4 | ..... | 72·4 | 4·2 | 16·2 | 58·7 | 14·9 |
| | | (7) | (7) | (7) | | (7) | (7) | (7) | (7) | (7) |

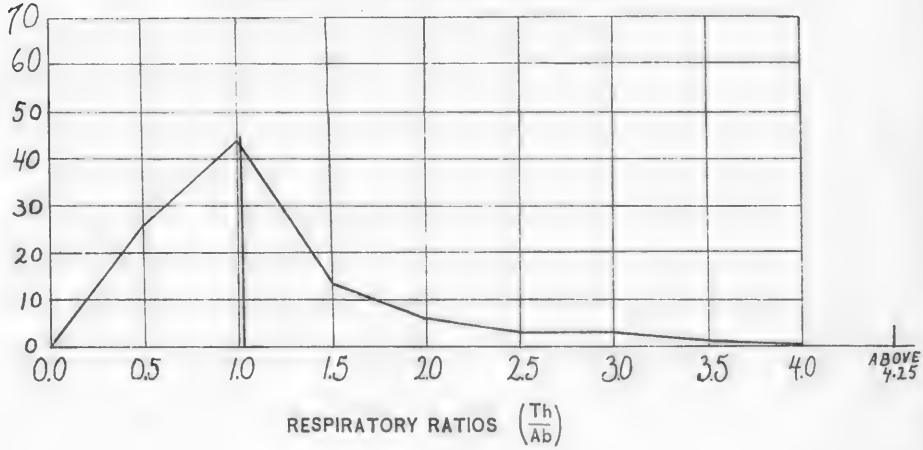
DESCRIPTION OF PLATES XXXIV, XXXV, AND XXXVI.

See text (p. 680 et seq.).

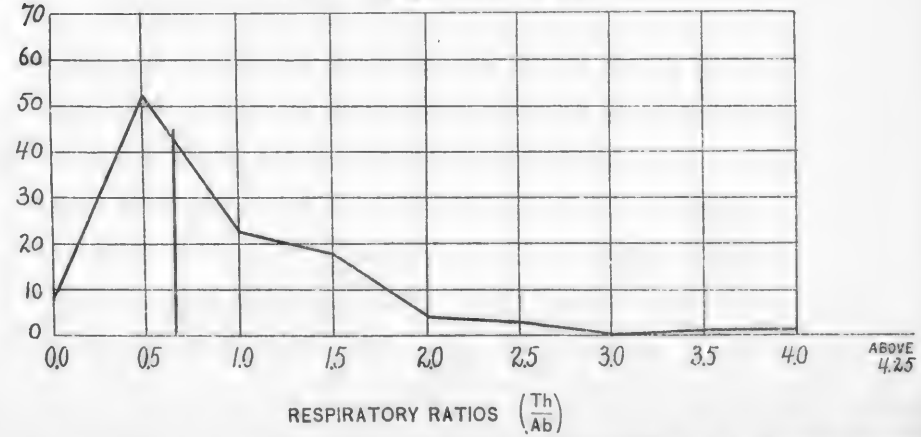
CURVE I. 35 SCHOOL BOYS.



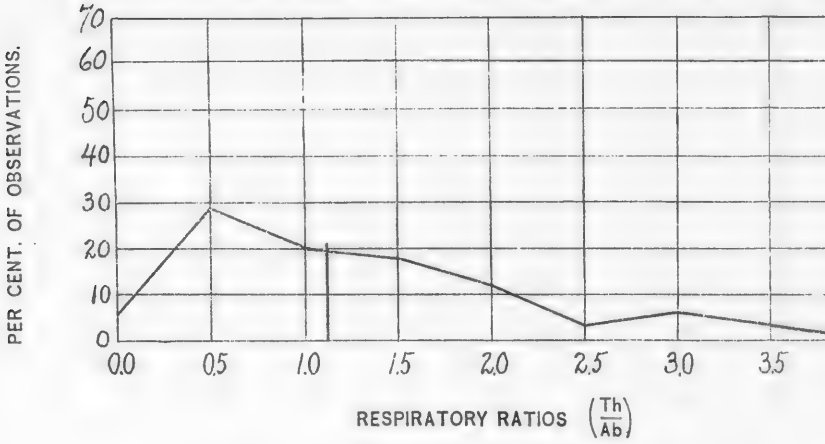

CURVE II. 72 SCHOOL GIRLS.



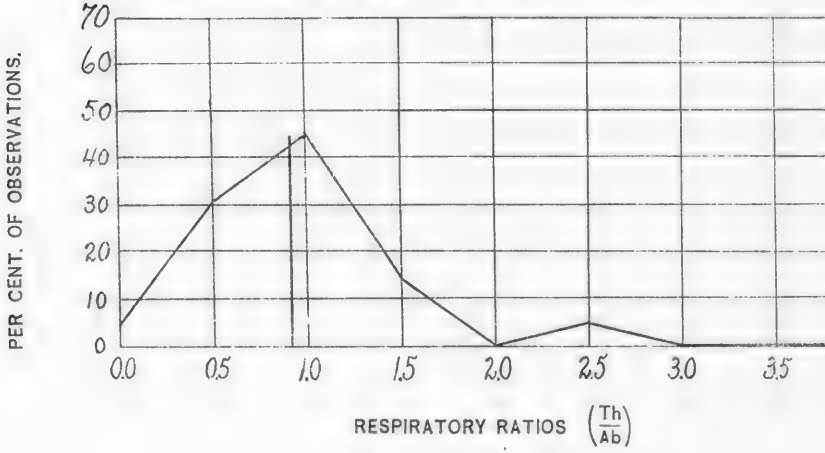

CURVE III. 124 COLLEGE MEN.



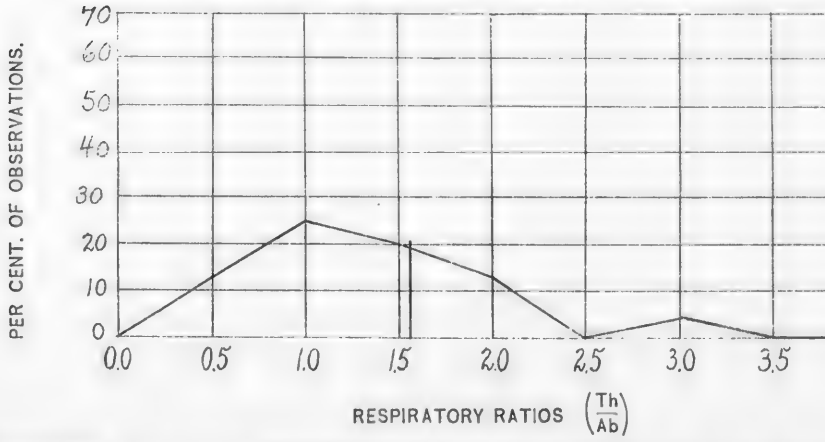
CURVE IV. 34 FEMALES, UNCONSTRICTING DRESS.




CURVE V. 22 MALES, UNCONSTRICTING DRESS.



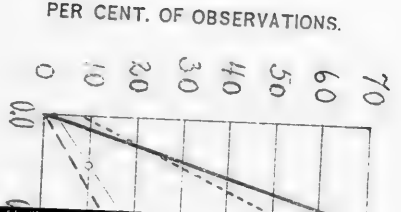

CURVE VI. 24 MALES, CONSTRICTING DRESS.

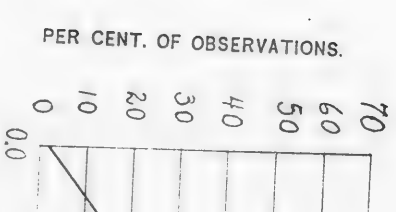


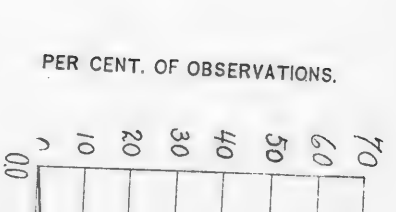
4.0
ABOVE
4.25

4.0
ABOVE
4.25

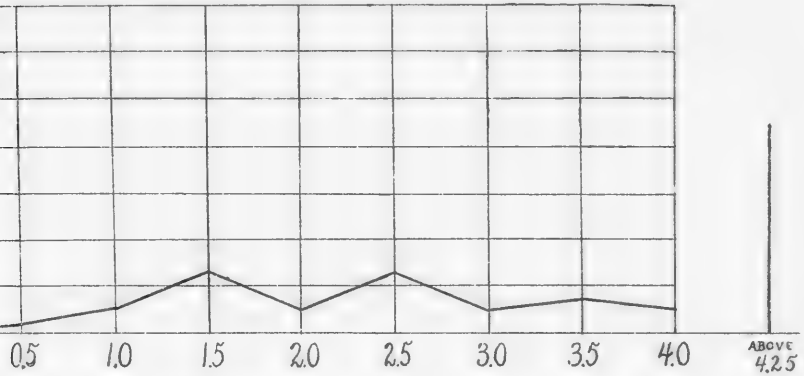
4.0
ABOVE
4.25


PER CENT. OF OBSERVATIONS.
0
10
20
30
40
50
60
70
0.0


PER CENT. OF OBSERVATIONS.
0
10
20
30
40
50
60
70
0.0

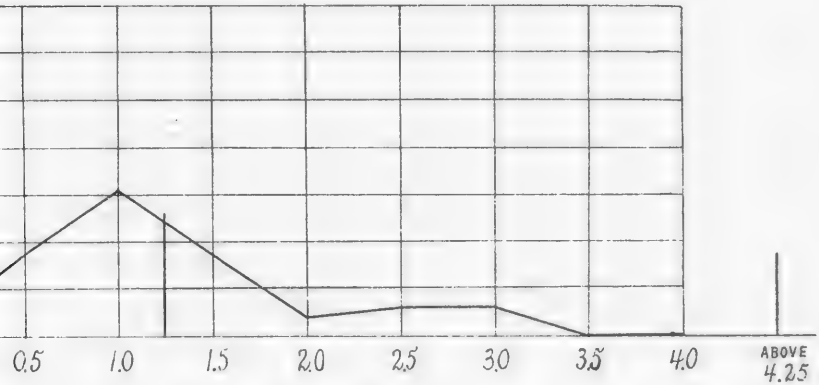

PER CENT. OF OBSERVATIONS.
0
10
20
30
40
50
60
70
0.0

CURVE VII. 44 FEMALES, CONSTRICTING DRESS.



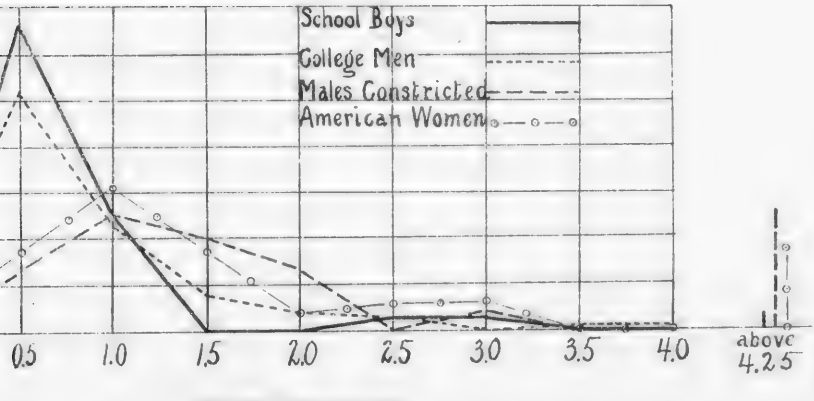

RESPIRATORY RATIOS $\left(\frac{Th}{Ab}\right)$

CURVE VIII. 52 AMERICAN WOMEN.




RESPIRATORY RATIOS $\left(\frac{Th}{Ab}\right)$

CURVE IX.




RESPIRATORY RATIOS

UNIV. OF MICH.

# THE IMMUNIZING POWER OF NUCLEOHISTON AND OF HISTON.

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## Historical.

In 1892 Lilienfeld * showed that the nuclei of the lymphocytes of the thymus gland contained a characteristic body, which he first termed leuconuclein. A subsequent study revealed that this compound was not a true nuclein, but that it contained, in addition to nuclein, another body—histon. It was accordingly renamed as nucleohiston. The nuclei of cells, therefore, contain a very complex proteid compound—nucleohiston—which contains nuclein and histon in a saltlike combination.

Before Lilienfeld's work several investigators undoubtedly experimented with what was more or less pure nucleohiston. Thus, Wooldridge's tissue fibrinogen, which he obtained from the thymus and the testicle, is largely nucleohiston. Wooldridge prepared his tissue fibrinogen by precipitating with an acid the aqueous extract of the gland. The precipitate thus obtained was washed and finally dissolved in dilute sodium hydrate or carbonate. This method, it will be observed, is essentially the same as that employed subsequently by Lilienfeld. As is well known, with this tissue fibrinogen Wooldridge obtained some unique results.

In the first place, an injection of the alkaline solution of tissue fibrinogen intravenously in rabbits produced total thrombosis of the venous system. In dogs, however, this thrombosis was observed only

* The references to literature cited in the text are at the end of this article.

in special places, such as the track of the portal vein, and usually recovery took place. In the animals that recovered a much larger amount could be injected a second time without effect. This condition of non-coagulability as a result of the first injection was observed to persist for several days. When boiled, the fibrinogen solution lost the power of producing coagulation within the blood vessels, although it still coagulated pepton-plasma. These results have been confirmed and enlarged by Lilienfeld in his work with nucleohiston. The two components of this compound, however, were shown to possess opposite and antagonistic properties. Thus, nuclein promptly induced coagulation intravenously or in extravascular blood, such as pepton-plasma. Histon, on the other hand, injected intravenously, produced a non-coagulable condition of the blood.

With his fibrinogen solution Wooldridge further obtained some results on immunity from anthrax which at the time attracted no little attention. According to the degree of alkalinity, the fibrinogen solution on boiling either coagulated or became opalescent, or was scarcely altered. The last result was obtained with strongly alkaline solutions. In such the anthrax bacillus grew well, but, when filtered through paper and injected intravenously into rabbits, failed of effect. In the feebly alkaline solutions, which therefore were opalescent or partly coagulated, the anthrax germ grew poorly; but when such cultures were sterilized by heat and injected into the veins of rabbits, immunity resulted in eight out of nine animals. Subcutaneous injection of the sterilized fibrinogen culture fluid did not protect against subcutaneous anthrax, whereas intravenous application did. In another series of experiments he obtained immunity in a limited number of animals by the injection of boiled fibrinogen solution wholly devoid of bacterial products. This was one of the first instances where immunity was secured with a non-bacterial substance. The fibrinogen solution was boiled slightly, so that when squeezed through muslin an opalescent fluid with suspended particles resulted. With such liquids Wooldridge immunized two rabbits from anthrax.

These experiments of Wooldridge are not free from objections. The experimental animals were rabbits, which, as is well known, are

held by many investigators to differ greatly in their susceptibility to anthrax. This difference, however, is denied by Baumgarten. The injection was made apparently by subcutaneous application, which renders the dose employed still more uncertain. Lastly, the control animals, instead of being of the same species, were guinea-pigs, the most susceptible of all animals to anthrax.

After Wooldridge's death, Wright (1891) experimented along the same line, with confirmatory results. He designated the "tissue fibrinogen" as cell fibrinogen, and showed that it yielded on decomposition an albumose body and a nuclein. After injections of this cell fibrinogen, the number of leucocytes in the blood was increased, and the albumose or pepton body appeared in the urine. It may be mentioned here that histon has been reported in febrile urine (Krehl and Matthes, 1895) and in leukæmic urine (Kolisch and Burián, 1896).

The solution of the cell fibrinogen in one-per-cent sodium carbonate was filtered through paper, and afterward through porcelain. This method of sterilization was resorted to in preference to the heating method of Wooldridge. Repeated intravenous injections (2 to 4 cubic centimetres at a time), before and after inoculation with anthrax, gave apparent immunity to four out of fifteen rabbits. Unfortunately, one out of four control rabbits survived. This recovery of a control is hardly due, as Wright was inclined to believe, to a washing away of the germ by a "hæmorrhage at the point of inoculation." As in Wooldridge's experiments, no mention is made of the method of inoculation or the quantity, source, etc., of the culture employed. The crucial test of an established immunity—reinoculation with an otherwise surely fatal dose—was not performed. The life of the treated animals was certainly prolonged (four, five, and six days), but no evidence is brought forward to show that this is not due to the alkalinity of the solution employed.

Brieger, Kitasato and Wassermann, in 1892, attempted to immunize white mice and guinea-pigs from anthrax by a similar process. Their results show a partial immunity in a limited number of instances, but not one of the animals was proof against such

highly virulent material as a bit of the spleen of an animal dead of anthrax.

These authors obtained much more satisfactory results with other pathogenic bacteria. Mixtures of tetanus culture and thymus extract on standing showed diminished toxicity, and mice and rabbits treated with successively increasing doses of such mixtures eventually became perfectly immune from tetanus. The thymus extract not only greatly diminished the production of tetanus toxin, but also favoured its destruction. Cultures of the diphtheria bacillus on thymus bouillon likewise possessed very weak toxic properties, and, after heating fifteen minutes at 65° to 70° to destroy the toxin, conferred immunity from diphtheria in the majority of the guinea-pigs experimented upon. The cholera vibrio grew well in thymus bouillon, and the cultures possessed considerable toxicity. By heating fifteen minutes at 65°, this toxicity was destroyed, and immunity was imparted to guinea-pigs treated with such heated cultures in eighty per cent of the animals experimented on. Similar results were obtained with typhoid cultures in thymus bouillon.

The supposition, apparently justified from these experiments, that the thymus extract possesses specific antitoxic properties is, as pointed out by Behring, erroneous. Modifications in the virulence of a culture must be expected when it grows on such an unfavourable medium as the thymus gland, and, as far as the immunizing results are concerned, subsequent investigations have firmly established the fact that this condition can be obtained by the more or less modified specific toxin. That the toxins can be modified or destroyed by nucleohiston will be seen from the work to follow.

The recent advances in our knowledge of the chemistry of the cell, and more especially of "tissue fibrinogen," made it desirable to investigate the protective power, if any existed, of nucleohiston and of its components histon and nuclein with reference to pathogenic bacteria as well as to bacterial toxins. As already stated, Brieger, Kitasato and Wassermann experimented with what was virtually nucleohiston. The work thus far has covered nucleohiston and histon, and this paper deals with these two compounds.

### Nucleohiston.

*Preparation.*—The method employed was essentially that of Lilienfeld, with such modifications as the means at my disposal necessitated. The perfectly fresh glands, when carefully freed of fat and connective tissue, weighed 10.65 kilos. The glands were cut up by a small sausage machine, and the soft pulp then put through an Enterprise fruit press. The thoroughly expressed connective tissue, etc., amounted to 1,180 grammes. The soft pulp, consisting largely of lymphocytes, was treated with eight to ten volumes of water, and allowed to stand in a very cold room overnight. It was then treated with acetic acid as long as a precipitate continued to form. The precipitate was allowed to settle twenty-four hours, and then the supernatant liquid was removed with a siphon. The precipitate was then washed twice by decantation with a very large quantity of distilled water. The compact pure white preciptate was now treated with an equal volume of a one-per-cent solution of sodium carbonate. It dissolved promptly, but the nucleohiston toward the end of the operation is not so readily soluble. The filtration of the alkaline fluid began in about an hour. On account of the slimy matter present and the large volume of liquid to be filtered (about 100 litres), filtration through paper was not resorted to. Instead an asbestos filter was employed. This was prepared as follows: A Witte's perforated porcelain plate (8 centimetres in diameter) was placed in a funnel, covered with a thin layer of wet muslin; then suction was applied by means of a Chapman aspirator, and suspended asbestos was poured into the funnel. A second layer of muslin was then placed on top, and on this another thin layer of asbestos, and finally a third layer of muslin. As soon as the filter began to clog in the process of filtration, which was assisted by an aspirator, gentle rubbing of the surface layer of muslin with the fingers or a spatula was found to greatly diminish this difficulty. Eventually when the filter did clog, the upper layer of muslin and asbestos was removed, and the lower layers were found to continue the filtration with equally good results.

The clear filtrate thus obtained was treated with acetic acid to

distinct and permanent acid reaction. After settling twenty-four hours, the clear liquid was siphoned off and the precipitate was then washed twice by decantation with large quantities of distilled water. The subsided precipitate was then dissolved in an equal volume of one-per-cent sodium carbonate. This solution could now be filtered through Schleicher and Schüll No. 580 plaited paper without any very great difficulty. A large number of funnels and frequent change of filter paper were all that was necessary. The clear filtrate, on acidification with acetic acid, gave a pure white precipitate, which was allowed to subside and then washed as above. The precipitate was finally transferred to plaited filters (No. 580) and allowed to drain. It was now scraped carefully from the filters and treated with several volumes of absolute alcohol. After standing overnight, the alcohol was decanted and replaced with an equal volume of alcohol and ether. The alcohol-ether liquid was finally decanted, and the precipitate was transferred to a muslin bag and allowed to drain. It was finally spread on previously heated porous porcelain plates and allowed to dry in the air. The dried mass was pulverized and passed through a fine copper sieve. The total yield of the air-dried product from 10.65 kilos of dressed glands was 717 grammes (6.7 per cent).

The following analysis will show the composition of the product prepared by this method. The sample was first extracted in a Soxhlet apparatus with absolute alcohol, then with ether, and finally was dried at 110° to 120° to constant weight.

*Sulphur and Phosphorus Determinations.*—The oxidation of the organic matter was effected after Hammarsten's method. The fused mass was dissolved in water, acidified with 5 cubic centimetres of nitric acid, evaporated to a small volume (about 100 cubic centimetres), and precipitated with barium nitrate. The filtrate and wash water from the barium sulphate was again concentrated, and finally precipitated with ammonium molybdate solution at a temperature of about 60° C. This phosphomolybdate precipitate was then washed as usual with acid ammonium nitrate, dissolved in dilute ammonium hydrate and precipitated with magnesia mixture.

*a.* 1.0162 grammes of substance gave 0.5073 gramme $BaSO_4 = 0.69$ per cent S.; also 0.10263 gramme of $Mg_2P_2O_7 = 2.82$ per cent P.

*b.* 1.0328 grammes of substance gave 0.05113 gramme $BaSO_4 = 0.68$ per cent S.; also 0.10483 gramme of $Mg_2P_2O_7 = 2.83$ per cent P.

*Nitrogen Determination.*—This was determined in the usual manner by Kjeldahl's method.

*a.* 0.3181 gramme of substance neutralized 38.1 cubic centimetres of $\frac{N}{10}$ oxalic acid (corrected for blank). The calculated amount of nitrogen is 0.5334 gramme = 16.77 per cent N.

*b.* 0.3076 gramme of substance neutralized 36.55 cubic centimetres of $\frac{N}{10}$ oxalic acid (corrected for blank). The calculated amount of nitrogen is 0.5117 gramme = 16.63 per cent N.

| | Lilienfeld. | Brieger, Kitasato and Wassermann. | Novy. |
|---|---|---|---|
| S. per cent. | 0·70 | ... | 0·69 |
| P. " | 3·02 | 2·9 | 2·83 |
| N. " | 16·86 | ... | 16·70 |

When ignited in a platinum dish, it yielded 2.78 per cent ash, which contained chiefly phosphoric acid and iron. The presence of iron in nucleohiston was pointed out by Wooldridge and by Lilienfeld. Brieger, Kitasato and Wassermann obtained 1.07 per cent ash from their "active principle" of the thymus gland.

The *chemical properties of nucleohiston* may be briefly summarized: When freshly precipitated and still moist, it is readily soluble in excess of acetic acid (also in 0.4 per cent HCl, Wright), in dilute neutral salt solution, in dilute sodium carbonate, sodium phosphate, sodium hydrate, in dilute acids, ammonium hydrate and ammonium sulphide. According to Lilienfeld, as well as Krehl and Matthes, on treatment with alcohol it becomes insoluble in sodium carbonate, in salts and in acids. This is true of the air-dried nucleohiston, whether treated with alcohol or not. In this condition it can be readily brought into solution by the addition of a few drops of sodium hydrate.

Nucleohiston is insoluble in water, alcohol, ether and benzol; the neutral solution or even the sodium-carbonate solution is precipitated by carbonic acid; also by acetic and hydrochloric acids, platinum chloride, silver nitrate and mercuric chloride. The solution is not precipitated with magnesium sulphate or sodium chloride, nor does it gelatinize with sodium hydrate or salts. The solution in sodium hydrate or carbonate treated with alcohol gives a precipitate of the sodium compound which is readily soluble in water, and treated with barium chloride gives the barium compound.

Nucleohiston gives the Millon and xanthoproteic reactions, also a faint biuret reaction on standing. It decomposes peroxide of hydrogen, but this property is lost on treatment with alcohol and ether. Digested with pepsin-hydrochloric acid, it yields an abundant precipitate of nuclein, containing 4.99 per cent of phosphorus. On boiling with water, it yields a nuclein, which is soluble, and histon. Treated with calcium hydrate, a precipitate of Ca-histon forms, whereas the nuclein remains in solution. Barium hydrate, on the other hand, gives a precipitate of barium nuclein, and the histon remains in solution. The nucleohiston is also readily split up by the action of acids, and, if heated, yields nucleinic acid and, finally, adenin, guanin, cytosin, etc.

The action of nucleohiston on the blood in rabbits and dogs has been referred to. I have been able to obtain similar results in rabbits. Krehl and Matthes found that a subcutaneous injection of 0.5 gramme of nucleohiston (unlike albumose) in guinea-pigs produced no fever. The same dose in tubercular guinea-pigs was also without effect on the temperature, but death followed in a short time with or without a local reaction.

Contrary to the above statements, I have found that intravenous or subcutaneous injections of 0.3 gramme of nucleohiston produce in rabbits (to a less extent in guinea-pigs), after a temporary subnormal temperature, a rise of 1° to 1.5° C. The febrile condition may last several days. After several injections, the animals scarcely react.

According to Freund and Grosz, nucleohiston precipitates the diphtheria toxin quantitatively, and in this respect resembles nucleinic

acid. It also is said to precipitate antitoxin from serum. This precipitation of toxin or of antitoxin is clearly not direct, but rather a mechanical dragging down of these active constituents in the same way as this is done by a precipitate of calcium phosphate. The addition of hydrochloric acid as practised in both cases results in the precipitation of nucleohiston, and with this are carried down the substances mentioned. This criticism also applies to Tichomiroff's precipitation of toxins with nucleinic acid, which readily forms a precipitate with proteids.

### Nucleohiston and Tetanus Toxin.

The powdered nucleohiston, as already stated, is with difficulty soluble in water. A strong aqueous solution can not, therefore, be prepared directly. The procedure followed in this and other instances was to add a few drops of strong potassium-hydrate solution to the suspension of nucleohiston in water. The compound dissolves quite easily, especially on standing. The solution of nucleohiston is then filtered and precipitated by slightly acidulating with acetic acid. The precipitate is allowed to settle and the liquid decanted. The precipitate is then washed once or twice by decantation, and finally transferred to a filter. As soon as the liquid has drained off, the moist precipitate is stirred into a one-per-cent solution of sodium carbonate containing a few drops of alkali. Freshly precipitated nucleohiston dissolves quite readily in sodium carbonate. In this way a ten-per-cent milky opalescent solution can be obtained. For use, this was usually diluted with an equal volume of distilled water, thus giving a five-per-cent nucleohiston solution in 0.5-per-cent sodium carbonate. Any free alkali present is fixed by passing carbonic acid through the solution until it begins to yield a precipitate.

The tetanus toxin was prepared by filtering the culture of the bacillus in glucose bouillon through porcelain. A minute amount of tricresol was added to the toxin.

Only guinea-pigs were employed in the work with tetanus toxin. The injections were made subcutaneously and separately, with but a slight interval of time between. Guinea-pigs 56 and 57 received one

half of the nucleohiston intraperitoneally, the other half subcutaneously.

TABLE I.

| Guinea-pig No. | Weight. | Nucleohiston. | Tetanus toxin. | Death, in hours. |
|---|---|---|---|---|
| 55 | 290 | 250 mg. | 0·1 c. c. | 50 |
| 56 | 310 | 500 " | 0·1 " | 40 |
| 57 | 375 | 500 " | 0·1 " | 50 |
| Control 58 | 416 | ... | 0·1 " | 46 |

The nucleohiston, therefore, under these conditions does not protect against tetanus toxin.

In the following experiment, 0.5 cubic centimetre of tetanus toxin was mixed with 5 cubic centimetres of a five-per-cent nucleohiston solution; 1.1 cubic centimetres of the mixture, therefore, contained 0.1 cubic centimetre of tetanus toxin. This quantity of the mixture was injected subcutaneously in guinea-pigs at intervals indicated in the table. The mixture was kept in an ice chest.

TABLE II.

| Guinea-pig No. | Weight. | Dose. | Mixture used. | Death, in hours. |
|---|---|---|---|---|
| Control 58 | 416 | 0·1 c. c. toxin. | ..... | 46 |
| 59 | 325 | 1·1 " mixture. | At once. | 50 |
| 60 | 200 | 1·1 " " | After 24 hours. | 44 |
| 61 | 265 | 1·1 " " | " 48 " | 48 |
| 62 | 250 | 1·1 " " | " 96 " | No effect. |

From this it is seen that the nucleohiston solution does not weaken or destroy the tetanus toxin until after contact for two days. The mixture four days old failed to kill the guinea-pig, but conferred no immunity, since when inoculated eight days later with 0.1 cubic centimetre of tetanus toxin the animal died in thirty-two hours. On comparing this table with Table IV, it will be seen that the diphtheria toxin is destroyed by the nucleohiston solution much more rapidly than is the tetanus toxin. A similar relative weakness of the diphtheria toxin to formalin has been pointed out by Burckhard. The quantity of formalin sufficient to destroy the diphtheria toxin was not sufficient for the tetanus toxin. In other words, the tetanus toxin, which is often considered as the most unstable toxin, is with

reference to certain chemicals more permanent than the diphtheria toxin. The action of the nucleohiston solution is not due wholly, or even in great part, to the alkali present, as can be seen in the experiments with diphtheria toxin.

### Nucleohiston and Diphtheria Toxin.

In the following experiment the injections were made separately. Guinea-pigs 64 and 65 received two injections, each of 250 milligrammes of nucleohiston, one of which was given subcutaneously, the other intraperitoneally.

Table III.

| Guinea-pig No. | Weight. | Nucleohiston. | Diphtheria toxin. | Death, in hours. |
|---|---|---|---|---|
| 63 | 260 | 250 mg. | 0·1 c. c. | 80 |
| 64 | ... | 500 " | 0·1 " | 40 |
| 65 | ... | 500 " | 0·1 " | 40 |
| Control 66 | 230 | ..... | 0·1 " | 60 |

As in Experiment I with tetanus toxin, nucleohiston is without any immunizing or protecting action toward the diphtheria toxin.

In the following table are tabulated the results obtained with a mixture of 5 cubic centimetres of nucleohiston and 0.5 cubic centimetre of diphtheria toxin; 1.1 cubic centimetres of this mixture contained 0.1 cubic centimetre of diphtheria toxin. The mixture was kept in the ice chest. The injections were made under the skin.

Table IV.

| Guinea-pig No. | Weight. | Dose. | Mixture used. | Death, in hours. |
|---|---|---|---|---|
| Control 53 | ... | 0·1 c. c. toxin. | ..... | 49 |
| 67 | 285 | 1·1 " mixture. | At once. | 64 |
| 68 | 225 | 1·1 " " | After 24 hours. | No effect. |
| 69 | 245 | 1·1 " " | " 48 " | " |
| 70 | 250 | 1·1 " " | " 96 " | " |

From this it is seen that the nucleohiston solution destroys the diphtheria toxin in less than twenty-four hours. In the case of tetanus, as seen in Table II, the toxin is destroyed after the forty-eighth and before the ninety-sixth hour. This shows, as already pointed out, that the tetanus toxin is more resistant than the diphtheria

toxin to the action of nucleohiston. The animals (68, 69, 70) that survived the injection were not rendered immune, since an injection with 0.1 cubic centimetre of diphtheria toxin, made eleven, ten, and eight days respectively after the first injection, caused death in forty-eight, thirty-two, and forty-eight hours respectively.

This destruction of the tetanus and diphtheria toxin by the nucleohiston solution may be due in part to the sodium carbonate. The alkali, however, in double the strength employed weakens the toxin much more slowly than does the nucleohiston solution. This action of sodium carbonate can be seen from the following experiments. For this trial 0.8 cubic centimetre of diphtheria toxin was added to 4 cubic centimetres of a one-per-cent solution of sodium carbonate; 1.2 cubic centimetres of the mixture therefore contained 0.2 cubic centimetre of toxin. The first animal was inoculated after the mixture had been allowed to stand for one hour. The latter was then placed in the ice chest and inoculations were made as indicated.

TABLE V.

| Guinea-pig No. | Weight. | Dose. | Mixture used. | Death, in days. |
|---|---|---|---|---|
| Control 71 | 420 | 0·1 c. c. toxin. | ..... | $2\frac{1}{4}$ |
| " 72 | 514 | 0·2 " " | ..... .. | $3\frac{1}{3}$ |
| 78 | 422 | 1·2 " mixture. | 1 hour old. | $1\frac{2}{3}$ |
| 79 | 395 | 1·2 " " | 52 hours old. | $7\frac{1}{4}$ |
| 90 | 244 | 1·2 " " | 100 " " | No effect. |

This shows that while a nucleohiston solution containing one half per cent sodium carbonate destroys the toxin within twenty-four hours, a one-per-cent sodium-carbonate solution does not destroy the toxin, but weakens it in about two days. The toxin used was the same as that employed before, but was weakened by age; hence a larger dose was used.

### NUCLEOHISTON AND HOG CHOLERA.

The bouillon cultures of the hog-cholera bacillus employed for inoculation were always from two to three days old. The injection of nucleohiston was in some cases repeated two or three times at intervals of two days.

TABLE VI.

| Rabbit No. | Weight. | Nucleohiston. | Hog-cholera bouillon. | Death, in days. |
|---|---|---|---|---|
| 60 | 1,230 | 250 mg. intravenously. | 0·25 c. c. | $3\frac{1}{4}$ |
| 61 | 1,580 | 250 " subcutaneously. | 0·25 " | $6\frac{1}{2}$ |
| 62 | 2,000 | 400 " intravenously in 2 injections. | 0·25 " | 3 |
| 63 | 1,580 | 750 " subcutaneously in 2 injections. | 0·25 " | $5\frac{1}{2}$ |
| 64 | 2,100 | 1·0 g. intravenously in 3 injections. | 0·25 " | 4 |
| 65 | 1,800 | 1·25 g. subcutaneously in 3 injections. | 0·25 " | 5 |
| Control 66 | 1,550 | ..... | 0·25 " | $2\frac{1}{2}$ |

It would appear from this set that nucleohiston at least apparently retards death from the hog-cholera bacillus. Recognising, however, the variation that exists in the susceptibility of rabbits to this germ, the conclusion is justified that nucleohiston does not protect against hog cholera; 2.5 grammes of nucleohiston introduced into a rabbit (67) in four injections, three, twenty-four, thirty-six, and seventy hours after inoculation with 0.25 cubic centimetre of hog-cholera culture, did not prevent death, which ensued in three days and a half.

### NUCLEOHISTON AND ANTHRAX.

It has been pointed out in the introduction that with "tissue fibrinogen" or "cell fibrinogen" Wooldridge and Wright believed that they obtained immunity from anthrax. Wooldridge had apparent success in two and Wright in four cases. There is no evidence, as pointed out, that immunity really was established, since reinoculation was not resorted to. In the following experiments only one rabbit (No. 53) out of twelve animals was protected against the injection of an anthrax culture. On subsequent inoculation, however, eleven days later, the rabbit died of anthrax in three days and three quarters. This rabbit received intravenously 400 milligrammes of nucleohiston. During the next twenty-four hours the temperature rose to 40.5°. On the second day it returned to the normal, 38.9° to 39.5°, and on the third it was subnormal, 37.3° to 38°. At this point the rabbit was inoculated with 0.1 cubic centimetre of a fresh bouillon culture of anthrax. Rabbits Nos. 51 and 52 were treated in exactly the same way. Their temperature before inoculation, however was 39.4° and 39.6°.

The injections were made separately, except in guinea-pigs 50

and 51, where a mixture of 0.3 cubic centimetre of anthrax culture and 2.7 cubic centimetres of a five-per-cent nucleohiston was employed. One cubic centimetre of this mixture therefore contained 0.1 cubic centimetre of the anthrax culture. The anthrax injections were in all cases made subcutaneously, and, unless otherwise indicated, were made immediately after the injection of the nucleohiston.

With the exception of Rabbit 53, the results, as seen from Table VII, are sufficient to show that nucleohiston possesses no specific action with reference to anthrax.

TABLE VII.

| Animal No. | Weight. | Nucleohiston. | Anthrax. | Death, in days. |
|---|---|---|---|---|
| Rabbits. | | | | |
| Control 55 | 1,570 | ..... | 0·1 c. c. | $3\frac{1}{12}$ |
| 51 | 1,440 | 0·3 gm. intravenously. | 0·1 " 2 days later. | $3\frac{1}{2}$ |
| 52 | 1,389 | 0·5 " " | 0·1 " 2 " " | $3\frac{1}{2}$ |
| 53 | 1,590 | 0·4 " " | 0·1 " 2 " " | Recovered. |
| 56 | 1,200 | 0·675 gm. intravenously, 3 injections in 8 days. | 0·1 " 3 " " | $2\frac{1}{2}$ |
| 57 | 1,090 | 1 gm. subcutaneously, 3 injections in 8 days. | 0·1 " 3 " " | 3 |
| 58 | 1,050 | 0·5 gm. subcutaneously. | 0·1 " | 2 |
| 59 | 1,670 | 0·3 " intravenously. | 0·1 " | 2 |
| Guinea-pigs. | | | | |
| Control 47 | 824 | ..... | 0·1 " | $1\frac{1}{2}$ |
| 46 | 490 | 0·3 gm. subcutaneously. | 0·1 " 2 days later. | $4\frac{1}{4}$ |
| 48 | 600 | 0·5 " " | 0·1 " | $1\frac{3}{4}$ |
| 49 | 660 | 1 gm. subcutaneously, 3 injections in 8 days. | 0·1 " 3 days later. | $1\frac{1}{2}$ |
| 50 | 758 | 1 c. c. of anthrax-nucleohiston mixture, 4 days on ice. | ..... | $2\frac{1}{2}$ |
| 51 | 800 | 1 c. c. of anthrax-nucleohiston mixture, 11 days on ice. | ..... | 3 |

## HISTON.

The term histon was employed first by Kossel to designate an albumose or peptonlike body which he found in the red blood cells of geese. He showed that it did not exist as such in the cells, but in combination with another body, presumably nuclein. In 1891 Wright showed that "tissue fibrinogen" readily split up into an albumose half and a nuclein half. The injection of "tissue fibrinogen" in rabbits and dogs increased the number of leucocytes, and in the urine he found albumose or pepton. As already pointed out, histon has been found during the past year in febrile and leukæmic

urines. The researches of Lilienfeld have shown that "tissue fibrinogen" is largely made up of nucleohiston, which readily yields nuclein and histon.

Histon may be prepared from nucleohiston or from the gland direct. The nucleohiston (50 grammes) is rubbed up in a mortar with 250 cubic centimetres of 0.8-per-cent HCl (8 cubic centimetres of 1.20 specific gravity HCl to 1,000 cubic centimetres of water). After standing twenty-four hours, the liquid is decanted and filtered, and the filtrate precipitated with six to eight volumes of a mixture of equal parts of absolute alcohol and ether. The precipitate is filtered, washed with ether, then pressed between filter paper, and finally allowed to dry in the air. The dried residue, powdered, is pure white and chalky in appearance. The residue after the first extraction can be treated with the dilute hydrochloric acid several times in succession, and the filtrate from each treatment yields an additional quantity of histon. The product, however, was at first yellowish, waxy in character, and when dried gave a yellowish powder. The total yield of histon in this case was nearly 5 grammes.

The filtration in this method is very slow, and it is much more convenient to resort to centrifugation, in which case the yield of histon is better. The yield is usually about ten per cent of the air-dried, powdered nucleohiston.

Histon was also prepared direct from the fresh thymus glands. The glands, freed from connective tissue, etc., were passed through an Enterprise fruit press. The thick pulp thus obtained was treated with two volumes of water, and set aside at 39° for two hours, with frequent stirring. The slimy character is thus greatly diminished, and the material brought quickly into solution. Hydrochloric acid (one per cent) was then added, and the liquid stirred frequently during the next four hours. The mixture was then centrifugated, and the clear, opalescent liquid was decanted and filtered through paper. This liquid, precipitated with alcohol and ether, as in the preceding method, gave a yellowish, semi-crystalline powder. The yield was about 3.6 per cent of the fresh gland.

The *reactions* of histon can be briefly summarized. It is readily

soluble in water, and the slightly opalescent solution thus obtained is distinctly acid. The solution is precipitated on saturation with sodium chloride, sodium carbonate, ammonium chloride, ammonium sulphate, and magnesium sulphate. This latter fact is of importance, since it shows a relationship to the globulins which, as will be seen presently, possess a similar action on toxins. A white, flocculent precipitate is also given by ammonium hydrate. If the precipitate is allowed to subside and stand for a while, it becomes insoluble in excess of reagent, whereas if an excess of ammonia is added at once the precipitate that forms redissolves immediately. According to Kossel, this ammonia precipitate is insoluble, but it is soluble in HCl (Lilienfeld) and in acetic acid (Kolisch and Burián). Calcium- and sodium hydrate also yield precipitates. Barium hydrate does not give a precipitate. Nitric acid gives a precipitate which disappears on heating and reappears on cooling (albumose reaction). The concentrated solution is also precipitated by alcohol.

Histon is not precipitated by calcium or mercuric chlorides, neutral or basic lead acetate, sodium phosphate, acetic or sulphuric acids. On boiling, according to Kossel, there is no coagulation, whereas Lilienfeld and Kolisch and Burián state that it is coagulated. The coagulum is soluble in mineral acids. On prolonged heating with baryta, it yields leucin and tyrosin. It does not contain phosphorus.

It gives the biuret reaction in the cold. This fact, together with its behaviour to nitric acid and its peculiar action on blood, led Lilienfeld to consider this body as an albumose. Its behaviour to magnesium sulphate and to heat shows a relation to globulins.

As is well known, Schmidt-Mülheim, and later Fano, showed that intravenous injections of 0.3 gramme of pepton, per kilo body weight, prevent coagulation of blood. Pollitzer eventually demonstrated that this property does not belong to true pepton, but rather to heteroalbumose. Intravenous injections of histon, as pointed out by Lilienfeld, give a similar non-coagulable condition of the blood. Histon-plasma, however, is different in many respects from pepton-plasma. With the histon prepared as above, I have been able to obtain characteristic histon-plasma as described by Lilienfeld. Histon, however,

is quite poisonous, and this toxic action is not due to the acid reaction of the solution, as Lilienfeld is inclined to believe. It is possible to inject intravenously, with only temporary effects, into rabbits many times the amount of free HCl corresponding to the acidity of the histon solution.

The several preparations of histon possessed, as stated, decided toxic properties. In guinea-pigs weighing 300 grammes or under, 100 milligrammes frequently produced marked intoxication, whereas 200 milligrammes often produced death. Repeated injections of small doses failed to establish a tolerance. Indeed, an increased susceptibility was observed in animals after two or three injections, so that a dose of 100 milligrammes eventually sufficed to produce death in a few hours. In animals that recovered from larger injections, a necrotic area would form at the point of inoculation.

Subcutaneous injection in guinea-pigs of 200 milligrammes produced a slight temporary depression of temperature, giving place in a few hours to an increase of 1° to 1.5° C., which would persist for forty-eight hours or longer.

Exceedingly rapid effects were brought out in rabbits with intravenous injections of histon. In this case a dose of 100 milligrammes of histon, whether acid or neutral, was dangerous, and often produced death in a few minutes. In such cases the heart was very irregular, respiration laboured, and the animal would lie in a helpless condition, with eyes moist, and often with symptoms of salivation.

The histon prepared according to the methods given is probably far from being a chemically pure product. The white chalky powder gave 0.7 per cent ash, whereas the histon prepared directly from the gland by the second process gave 1.45 per cent ash, which contained some chlorine and much phosphoric acid. This is further seen in the differences in reactions between the preparations made by the several workers and in the wholly unlike results obtained by experimentation on animals. The anti-coagulating power appears to be constant; but, on the other hand, apparently irreconcilable results have been obtained as to toxicity, as to thermogenic effects, and, above all and of most importance, as to immunizing properties. The immunizing

power of nucleohiston, as described by Wooldridge and by Wright, has been shown from the preceding work not to exist. The same may be said, in advance, to be true of histon.

The most noteworthy contributions in this direction have been made by Freund, Grosz and Jelinek. These authors have endeavoured to show a relation between blood coagulation and the action of antitoxins; in other words, that histon, which possesses anticoagulating powers, is likewise antitoxic in action. A single subcutaneous injection of 100 milligrammes or more of histon was sufficient to protect against a fatal dose of diphtheria toxin (0.2 cubic centimetre). Likewise an injection of 400 milligrammes of histon, followed twenty-four hours later by an injection of 0.15 cubic centimetre of a two-day diphtheria culture, was without effect, although the control died in two days. My experiments with histon have failed to confirm not only this protective action against the toxin, but also the harmlessness of injections of 400 to 500 milligrammes of histon. Half that amount, indeed, is sufficient, when introduced subcutaneously, in guinea-pigs, to produce death in a few hours.

Freund and Grosz, in a recent paper, record similar results with albumoses as with histon. Thus, an injection of 100 to 210 milligrammes of deuteroalbumose, or of 50 to 100 milligrammes of protalbumose, sufficed to protect against a fatal dose of diphtheria toxin. Ten milligrammes of either deuteroalbumose or protalbumose protected mice against a fatal dose of tetanus toxin (0.005 cubic centimetre), whereas 100 to 150 milligrammes of protalbumose protected rabbits against the same poison. It is needless to say that results like this, obtained with such small quantities of albumose, are open to question. Witte's pepton, which is largely made up of albumose, can be expected to give similar results, but thus far they have not been satisfactory.

In this connection attention may be called to the somewhat similar work of Smirnow, who found that 0.02 cubic centimetre and upward of a 1.1-per-cent solution of globulin from a normal serum of a horse, mixed with a fatal dose of diphtheria toxin, neutralizes the poison. Such a mixture when injected is without effect, but if the

globulin and the toxin be injected separately in different parts of the body, death follows. These results are not above suspicion, for it is altogether likely that something more than 0.0002 gramme of globulin is necessary to protect against the diphtheria toxin.

In the work with histon, as a rule, a ten-per-cent solution was employed. The histon solution is distinctly acid, 100 milligrammes requiring about 0.5 cubic centimetre of a one-per-cent solution of sodium carbonate for neutralization. In some instances the histon solution was neutralized before injection. As in the preceding experiments, the injections of histon and of toxin were made separately, subcutaneously, and in different places, unless otherwise indicated. About four different preparations of histon were employed, without any difference in the results.

### Histon and Tetanus Toxin.

Table VIII.

| Guinea-pig No. | Weight. | Histon. | Tetanus toxin. | Death, in days. |
|---|---|---|---|---|
| Control 2 | 280 | ..... | 0·1 c. c. | 3 |
| 3 | ... | 0·1 gm. | 0·1 " | $6\frac{1}{4}$ |
| 4 | ... | 0·2 " | 0·1 " | $6\frac{1}{4}$ |
| 19 | 252 | 0·2 " * | 0·1 " | $3\frac{1}{2}$ |
| 20 | 330 | 0·1 " † | 0·1 " | 9 |
| 41 | ... | 0·1 " neutral. | 0·25 " | $1\frac{1}{2}$ |
| 42 | ... | 0·1 " " | 0·25 " | $1\frac{1}{2}$ |
| Control 43 | ... | ..... | 0·25 " | $1\frac{1}{2}$ |

* On each of the two following days after the injection of the tetanus toxin an additional injection of 0·2 gramme of histon was made.

† Two injections of 0·1 gramme each, as in the preceding.

The first five animals received an old tetanus toxin solution, of which 0.1 cubic centimetre closely approximated the minimum fatal dose. The last three received another and more recent toxin. The failure of histon to protect animals from an intoxication with the tetanus poison is thus shown.

A fifteen minutes' old mixture of the toxin and histon, whether acid or neutral, does not save guinea-pigs. Death occurs about the same time as with controls. In a similar mixture of diphtheria toxin and acid histon the toxin is destroyed within five minutes. This again shows that the diphtheria toxin is less resistant than the tetanus toxin.

### Histon and Diphtheria Toxin.

The following experiments with diphtheria toxin furnished the same result as with tetanus toxin. Separate injections of histon and of diphtheria toxin do not protect against the latter.

Table IX.

| Guinea-pig No. | Weight. | Histon. | Diphtheria toxin. | Death, in hours. |
|---|---|---|---|---|
| Control 6 | 200 | ..... | 0·3 c. c. | 24 |
| 7 | 115 | 0·1 gm. | 0·3 " | 29 |
| 8 | 297 | 0·2 " | 0·3 " | 27 |
| Control 11 | 252 | ..... | 0·1 " | 30 |
| 9 | 290 | 0·2 gm. in 2 injections. | 0·1 " | 41 |
| 13 | 274 | 0·3 " " 3 " | 0·1 " | 36 |
| 35 | 287 | 0·1 " | 0·06 " | 60 |
| 36 | 160 | 0·1 " | 0·06 " | 36 |
| Control 37 | 357 | ..... | 0·06 " | 72 |
| " 41 | 232 | ..... | 0·06 " | 36 |
| 44 | 185 | 0·1 gm. neutral. | 0·06 " | 36 |
| 45 | 170 | 0·1 " | 0·06 " | 36 |
| Rabbit 27 | Control | ..... | 0·5 " | 42 |
| " 29 | 1,560 | 0·08 gm. intravenously. | 0·5 " | 48 |
| " 36 | 1,920 | 0·18 " " | 0·7 " | 52 |

These results on rabbits and guinea-pigs are wholly unlike those of Freund, Grosz and Jelinek. In not a single instance was the animal's life prolonged, much less saved. Larger doses of histon than those given could not safely be employed, owing to the toxic action of the substance.

In the preceding experiments the injections of histon and of toxin were made separately in different parts of the body. As pointed out, the results are wholly negative, and consequently diametrically opposite to those of Freund, Grosz and Jelinek. A mixture of histon and toxin, however, when injected into guinea-pigs is not fatal, as the following trials indicate: The mixture was prepared by adding 0.8 cubic centimetre of the diphtheria toxin to 4 cubic centimetres of a ten-per-cent histon solution; 1.2 cubic centimetres of the mixture, therefore, contained 100 milligrammes of histon and 0.2 cubic centimetre of toxin, which represents a surely fatal dose. The toxin was the same as used in preceding experiments, but, owing to the lapse of several weeks, it had become less active. The histon solution was

decidedly acid, 1 cubic centimetre of the ten-per-cent solution requiring 1.2 cubic centimetres of $\frac{N}{10}$ KOH for neutralization. The injections were subcutaneous.

TABLE X.

| Guinea-pig No. | Weight. | Dose. | Age of mixture. | Death, in days. |
|---|---|---|---|---|
| 77 | 420 | 1·2 c. c. | 1 hour. | No effect. |
| 80 | 505 | 1·2 " | 52 hours. | " " |
| 85 | 505 | 1·2 " | 5 minutes. | " " |
| 87 | 510 | 1·2 " | 1 hour 40 min. | " " |
| Control 71 | 420 | 0·1 " toxin. | ..... | 2½ |
| " 72 | 514 | 0·2 " " | ..... | 3½ |
| " 89 | 535 | 0·2 " " | ..... | 2½ |

A mixture of the toxin and histon becomes non-poisonous within a few minutes. Is this failure of the toxin to act due to an immunizing action of the histon, or is it due to a direct action of the histon on the toxin, neutralizing or destroying the latter? Or, thirdly, is the toxin destroyed by the acidity of the histon solution? As for the first supposition, it is evident from the preceding experiments that histon solutions, either neutral or acid, fail to immunize. Furthermore, the animals that recovered from an injection of the mixture were not immune to a subsequent injection of toxin.

As for a direct action of the histon on the toxin, this is possible, especially if it be remembered that histon resembles albumoses and globulins in certain reactions. Ordinary albumoses and serum globulin of the horse have been reported to protect in mixture against the diphtheria toxin.

The strongly acid character of the histon solution must undoubtedly exercise a destructive action on the unstable diphtheria toxin. A mixture of 1.2 cubic centimetres of $\frac{N}{10}$ HCl and 0.2 cubic centimetre of the toxin, after standing one hour and forty minutes, was injected into a guinea-pig with no ill effects following. Indeed a similar mixture five minutes old is without effect. Furthermore, a neutral histon solution mixed with the toxin and injected five minutes later into a guinea-pig produced death in forty-four hours—the same time as for a control. It would appear from this that the destructive action of

histon on the diphtheria toxin is largely, if not wholly, due to the hydrochloric acid present.

### Histon and Hog Cholera.

In the following experiments fresh cultures in bouillon of the hog-cholera bacillus were employed. The injections were all subcutaneous:

Table XI.

| Rabbit No. | Weight. | Histon. | Hog cholera. | Death, in days. |
|---|---|---|---|---|
| 1 | 635 | 0·1 gm. neutral. | 0·1 c. c. | $4\frac{3}{8}$ |
| 2 | 940 | 0·2 " " | 0·1 " | $4\frac{1}{8}$ |
| Control 3 | 1,295 | ..... | 0·1 " | $2\frac{1}{2}$ |
| 4 | 1,020 | 0·8 gm. neutral, 5 inj. in 5 days | 0·1 " | $5\frac{1}{8}$ |
| 5 | 895 | 0·14 gm. | 0·1 " | 3 |

The results from histon injections in hog cholera are the same as those with nucleohiston.

In this connection we may add that unsuccessful attempts were made to produce immunity in rabbits with thymus bouillon cultures of the hog-cholera germ heated to 56°. The thymus bouillon culture therefore offers no advantage over the ordinary bouillon cultures.

### Histon and Anthrax.

The anthrax culture used for the following experiments was prepared and employed in the same manner as in the experiments with nucleohiston:

Table XII.

| Rabbit No. | Weight. | Histon. | Anthrax. | Death, in days. |
|---|---|---|---|---|
| 41 | 1,575 | 0·1 gm. intravenously. | 0·1 c. c. | $4\frac{1}{2}$ |
| 42 | 2,120 | 0·2 " " | 0·1 " | $3\frac{1}{2}$ |
| Control 43 | 835 | ..... | 0·1 " | $1\frac{5}{6}$ |
| 44 | 1,110 | 0·1 gm. neutral intrav. | 0·1 " | $1\frac{1}{2}$ |
| 45 | 1,550 | 0·1 " " " | 0·1 " | $2\frac{1}{2}$ |
| 46 | 1,590 | 0·1 " " " | 0·1 " | $2\frac{1}{2}$ |
| 48 | 1,800 | 0·1 " " subcut. | 0·1 " | $3\frac{1}{2}$ |
| 49 | 1,670 | 0·1 " " " | 0·1 " | $5\frac{1}{2}$ |
| Control 50 | 1,800 | ..... | 0·1 " | 3 |
| Guinea-pig 50 | .... | 0·1 gm. | 0·1 " | 2 |
| Control 51 | .... | ..... | 0·1 | $1\frac{1}{2}$ |
| 52 | .... | 0·15 gm. in 2 injections. | 0·1 " | $1\frac{1}{2}$ |
| 53 | 320 | 0·15 " " 2 " | 0·1 " | $1\frac{1}{2}$ |

The above table clearly shows that histon, like nucleohiston, is without any protective action against the anthrax bacillus.

## Conclusions.

The results obtained in the preceding experiments may be briefly summarized as follows:

1. Nucleohiston does not protect against a separate and subcutaneous injection of tetanus toxin, diphtheria toxin, hog-cholera bacillus, or anthrax bacillus.

2. Mixtures of nucleohiston and either tetanus toxin or diphtheria toxin lose their specific toxic action—the latter much more rapidly than the former. Animals that recover after inoculation of such mixtures are not rendered immune.

3. In a mixture of nucleohiston and anthrax bacillus the latter is unaffected, even after the lapse of eleven days.

4. The destruction of specific toxins in a solution of nucleohiston, though in part due to sodium carbonate, is probably chiefly due to the nucleohiston.

5. Histon does not protect against a separate and subcutaneous injection of tetanus toxin, diphtheria toxin, hog-cholera bacillus, or anthrax bacillus.

6. In a mixture of histon and diphtheria toxin the latter is destroyed in a few minutes. This action is in part, if not wholly, due to the acidity of the histon solution. Similar mixtures of closely related bodies, serum globulin or albumoses, in Witte's pepton, give analogous results. The animals that recover from such inoculations are not rendered immune. Histon does not destroy the tetanus toxin as readily as the diphtheria toxin.

7. Histon possesses decided and marked toxic properties which are not due to the hydrochloric acid present.

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